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## ANNOTATIONS, MINOR CONTRIBUTIONS, QUERIES

Under the above caption will be published from time to time comments criticisms and suggestions on technical procedures; minor contributions such as laboratory aids and short cuts which are not considered sufficiently important to warrant a formal paper; and queries.

Obviously comments and criticisms should be signed; queries should be signed but names will be withheld on request; full credit will be given those who contribute laboratory aids, short cuts and the like.

An attempt will be made to obtain answers from authoritative sources to the queries submitted. It must be emphasized that the views expressed in this department are not the opinions of any official body.

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# TECHNICAL, MECHANICAL, AND INTERPRETATIVE ASPECTS OF BASAL METABOLISM*

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Basal Metabolism is a subject concerning which much has been written pertaining to theory but one which has been almost wholly neglected from the angle of mechanics, graph censorship, and technique; so that the technician, however conscientious, has access to no satisfactory text wherefrom she can acquire an infallible technique and the clinician no instructive source of graph interpretation.

The average technician has such inadequate knowledge of the practical, mechanical, and technical fundamentals that many not only actually dislike the task of making an estimation, but also do not even know which of several repetitions of tests on the same individual should be accepted as accurate, nor why obviously erroneous results are such.

The average clinician or surgeon exhibits the same lack of instruction in his inability to censor for its degree of merited credence the kymograph chart as submitted. Most of them, unaware of the amount of reliable information inherent in the graph itself, accept the minus or plus value as indicated without scrutinizing the graph for possible flaws of mechanism or technique. Fortunately, however, one cannot be guilty of blind acceptance indefinitely.

The object of this article is to explain in a simple way the very important factors underlying the potentiality of an unquestionably reliable result in each individual case. It attempts to

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suggest a technique which guarantees accurate results for technicians and to provide a standard criterion of graph censorship for the busy doctor who must interpret the result.

The kymograph tracing should not be considered a complex, intricate hieroglyph nor set of hieroglyphics which requires the knowledge of an expert to interpret, but rather a very simple tracing which when scrutinized by the trained and observing eye proclaims aloud its own degree of merit. To be in a position to refer a patient for a test automatically makes one liable for the knowledge necessary to accept or reject the submitted result. Nor has the technician any prerogative whereby, because of unsatisfactory results, she may request the return of patients for repeated tests.

The physician who would have outstanding success in his metabolimetry must triply fortify himself. He must buy the very best and the most scientific machine manufactured. He must demand the services of an experienced and intelligent technician, and, last but not least, he himself must be able to interpret the graph. To omit or neglect any one of these three very important requisites is to fail; and to fail is unpardonable when to succeed is so easy.

Even thus triply fortified, one still may fail for the reason that the technician, however efficient, is forever confronted by new obstacles in the guise of psychological idiosyncrasies on the part of the patient which occasionally are capable of presenting themselves as almost insurmountable difficulties.

Average failure may not be excused under the name of, nor classed as that due to, psychological idiosyncrasy. Mental cases, a very sick patient, a very difficult one, or an unruly child with a behavioristic complex offer this type of difficulty.

Mechanical and technical aspects of basal metabolism are so closely related that, neither practically nor theoretically, can they be separated. Consequently the use of the most perfect machine is not a guarantee against unsatisfactory results unless operated by a qualified operator. Nor can an excellent technician with the magic wand of perfect technique be expected to obtain satisfactory results from an unqualified and unscientific mechanism.

Our study of and experience with metabolism testing have taught us to require certain qualifications of the instrument to be used. First of all it must be the product of scientific research. If it has been scientifically designed and constructed, the other necessary attributes usually take care of themselves. It must be equipped with some circulatory device other than an electrically driven blower; it should provide a kymograph cylinder propelled by an accurately timed kymograph clock, either mechanical or electrical; it must be designed to require a very large kymograph chart which allows for a long or a prolonged breathing time so that the patient may breathe for fourteen or fifteen minutes at one stretch without interruption; and finally it must be constructed to attack the problem of metabolism estimation from the proper angle, that of estimating the amount of oxygen consumed in a given length of time. It must not reverse this angle of attack by determining the length of time required to consume a given litre as such an attempt injects into the procedure an inverse variation of increased rate to decreased length of breathing time, especially when there is existent a condition of hyperthyroidism.

Whatever the circulatory device, it must be such that any impediment to breathing falls in the exhalatory half of the breath. The electric blower reverses this requisite. Flutter valves prove satisfactory. The flutter valve type of machine is so constructed that the bell is slightly overweighted increasing facility of breathing in favor of the inhalatory breath, the slight impediment introduced thereby into the exhalatory half makes no difference. The blower type with prolonged use reverts to a noise generator, in itself a deterrent from satisfactory results.

As to the kymograph cylinder, when properly supported at both base and top, the possibility of developing a leaning tower of Pisa effect is eliminated.

Only when a large kymograph chart is provided is flexibility of technique made possible; the section of the graph to be selected as representative of the basal rate may fall anywhere upon the chart, wherever the technician is best able to obtain it. In this contention I am bold enough to advance a theory heretofore not generally practiced, one which we have used successfully for over

fifteen years, that of not interrupting the patient at the end of an eight minute period, allowing him to rest and informing him that his second test must be taken.

Our reasons for this departure from the usual procedure are numerous. We are of the opinion that the psychology of the interrupted test is very bad, that it instills into the mind of the patient the impression that the first test was unsatisfactory and that therefore a second is being required.

Also at the end of eight minutes the patient only has gotten started. Why interrupt him until you have seen him establish his equilibrium and fall into his basal rate? When he has established it he will continue it as long as the chart holds out. Allow him to breathe a long time, and if the machine has been properly serviced the result obtained is unsurpassed for accuracy.

If he starts his test at an abnormal rate, hurries unnecessarily, breathes irregularly, or does any of the things we request him not to do, he soon tires himself out and cannot continue to breathe very long at a rate other than his basal; whereupon if left connected to the breathing tubes, he quickly falls into his equilibrium establishing his basal rate which is exactly what we want. The longer he breathes at one stretch the more certain the operator can be that the result obtained is correct. Prolonged breathing at a rate other than one's basal is as impossible as prolonged travel by foot, running instead of walking. After the first three or four minutes at the most almost any patient has settled down to his basal rate and continues without difficulty to the end of the chart.

The first three or four minutes, or more if they fall short of satisfaction, are to be disregarded in the final estimation, the six best consecutive minutes being chosen as the representative test. If there is a discrepancy of slope, the low value is usually the correct one. It is surprising to note how often the best part of the kymograph tracing falls upon the latter half of the chart. The patient actually breathes two tests instead of one, usually on exactly the same slope. In almost one hundred per cent of cases the graph slope when the basal rate has once become established remains the same throughout the entire length of the tracing.

Even though we are not requiring the graph in its entirety to fall on the same slope, it usually does. A discrepancy of slope after the first few minutes is the exception rather than the rule.

If the angle of attack is correct as determined by scientific design and construction, no factor which enters into the final estimation is limited or minimized by reason of the injection of an inverse variation; when reversed, the time element is decreased in proportion to the degree of hyperthyroidism existing. There must not enter into the procedure this inverse variation of decreased length of breathing time to increased rate of consumption; therein lies the mechanical defect of design.

With whom lies the responsibility? I shall not attempt to elucidate this point. I only can emphasize the fact that the reliability of any test is directly proportional to the length of time the individual is required to breathe, the longer the time, the more credible the result; the shorter, the less.

The fact that such an attempt eliminates the necessity of temperature and barometer corrections is no recommendation, as it sacrifices accuracy for ease and demands of the technician the intelligence of only a robot.

I am informed by the manufacturer of a scientific and classical machine that he is about to be obliged to change the character of his admirable kymograph chart and calculation system to simplify the calculation procedure to the method of placing a point here, another there, drawing a line, to behold the result precalculated; for no other reason than that the majority of technicians who use the apparatus are so generally uninformed that they do not know where to place the decimal point, unable to decide whether the result is .02 per cent or 20 per cent; .01 per cent or 10 per cent. This can be evidence only of very poor fundamental training on the part of the technician, as it requires the knowledge of only simple arithmetic to place it correctly. Inadvertently, it exposes the poor judgment exhibited by many admirable medical men in tolerating one who is not a technician. There are individuals well qualified to do outstanding work in this field. Certainly one who cannot place a decimal point should not be considered a technician. Until the members of the medical

profession become a little more cautious in referring their metabolism work to anyone who operates a machine they are destined to find themselves in the hands of unqualified persons who grind out results by predigested formula without the light of intelligence to guide them; and unfortunately the mistakes revert back, not upon the operator, but upon those who employ and tolerate her.

Two years college training plus adequate experience is not too much to ask in the way of preliminary preparation. A certain reliable clinic requires each prospective technician to watch at least five hundred tests given before she is allowed to attempt even one.

Mechanical ability on the part of the technician is an indispensable requisite. Before she attempts to run a test she must service her apparatus, not for any actual repair but to acquire a feeling that all is well and that any difficulty which may arise is not in any way to be attributed to a poorly or inadequately serviced mechanism, or to one whose mechanical make-up is not understood by the operator. If leaks develop she must be able to find and eliminate them, a process which calls for replacement of rubber tubing or only the readjustment of some part of the mechanism. Her inquisitiveness must lead her on until she is certain that she has found the difficulty and her mechanical ability assure her that she has corrected it. This preliminary servicing includes testing the absorptive power of the lime for possible exhaustion which amounts to nothing more than breathing into the machine herself to be certain that breathing is easy; for, if she cannot breathe into it herself she must know that the patient cannot either, and that the particular test is destined to certain failure. Difficulty of breathing usually is due to water being spilled into the breathing tubes or into the oxygen chamber, or to an exhausted lime supply. Other than this preliminary servicing there is no satisfactory method of determining the machine's efficiency. It is too late to discover or eliminate difficulty when the patient begins to experience it. Even the pen may refuse to write, or the kymograph mechanism fail to run. Such servicing should be done preliminary to each test.

The next exacting duty required of the technician is that of

instructing the patient in what he is to do and how he is to do it. This is one of the stumbling blocks as many technicians do not realize how great the need for a pedagogic or compelling psychology on her part, that the patient is in her hands for success or failure, and that what she gets from him is the product of her treatment of him. It is true he may present himself in the wrong frame of mind perturbed by fear and apprehension, but he is hers to instruct, hers to effect the accomplishment of a satisfactory result. Her psychology, attitude, instruction, manner, and powers of persuasion must be such that he has no chance to fail; and his reaction such that he knows he will not be allowed to fail. She must practically compel him tactfully and gently to give the correct result the first time he tries. She must take plenty of time to instruct him completely and minutely, to allay his fears, to help him keep in mind the few instructions he must remember. Some patients require persuasion and instruction almost to the point of hypnotism. I do not mean actual hypnotism but rather an induced confidence that he can and will give a coöperative and satisfactory test on what he should understand as the one and only test for the time being that it is intended he should have. The psychology of repeated tests to obtain a correct result is too unfortunate to tolerate. However, if during the first six or seven minutes he gives promise of complete failure without any suggestion of improving the character of his accomplishment during the seven remaining minutes allotted to him he must not be permitted to continue his failure on to the end. He must be disconnected with the admonition that the operator is sorry that he has forgotten some of the suggestions which will enable him to give a satisfactory test immediately, that she will give him several minutes to recover from the effort and instruct him more completely in the points to be remembered, so that he may continue and complete his test easily when she has substituted a fresh chart for the one which records a bad start.

It is legitimate from a psychological point of view to let him understand that he has started without "getting his better foot forward," but not legitimate to allow him to realize that he has

breathed the length of time of a required test. Upon a second start as a rule no difficulty arises, a reliable result is obtained and the psychology rescued. The necessity of a repeated test arises very seldom, less often than once a year. When handled in this way it is never necessary to take failure as the solution. Just as the grade school pupil is taught his elementary arithmetic whether he wants to learn it or not, so the metabolism patient the task before him. Thus the metabolism room becomes the school room wherein the individual is minutely and properly instructed and is not allowed to fail. If he is allowed to fail he probably will not return to you for his test, will hang the failure upon you, and seek out a more competent technician; he will have little faith in the procedure or examination, to say nothing of the diagnosis to be given him. This method of gentle pedagogic compulsion creates no feeling of resentment but rather a serious intention of mind and purpose to accomplish successfully the task in hand; and when the individual goes forth he is convinced that he has been in the hands of a master of the art.

When the technician has obtained the graph she must know that it is correct. She must have watched the patient throughout the test and have examined the nose and mouth connections before she disconnects them so that she can vouch for the fact that there has been no loss of oxygen at these points. She must have good mathematical training so that she does not fall into the class of the illiterate who cannot place a decimal point. Temperature and barometer corrections should not be beyond her power of comprehension.

The ideal metabolism room is equipped with a comfortable bed. It has a window to provide ventilation, in cold weather slightly open, in warm weather wide open to effect factually previous to the test facility of normal breathing. The room is cooled in summer and properly heated in winter so that the patient is comfortable at all times. The window shade is slightly drawn to suggest a quiet, cool, and restful atmosphere. Dark, unventilated, unpleasant, and uncomfortable surroundings are not conducive to good results. Previous to the test, exercise is

prohibited, and no breakfast given. The patient is required to remove shoes and tight clothing.

Certain instructions are given our patients to follow while taking the test. These they are not allowed to forget nor to disregard. Just before the test each patient is instructed to find a comfortable position and to remain physically quiet throughout the test. To induce mental quietude he is asked to close his eyes and is not allowed under any circumstances to keep them open. If he does not obey this last instruction a folded towel is laid gently over the trembling eyelids. He is asked or instructed to breathe as regularly and as naturally as he can for mouth breathing, not to hurry, and not to work, not to think very much about the test, but to continue to breathe in and out always taking as much air as he needs, avoiding as far as possible extremely long and repeated long breaths. He is given a few practice breaths before the nose clip is applied, the nose being held for a few seconds by the operator.

If a patient exhibits signs of nervousness, panic, fear, or failure to coöperate properly, he is not disconnected from the breathing tubes, but in a low, soothing tone of voice is told by the operator what he is not doing correctly; such as, "Remember to breathe naturally"—"Body still"—"Eyes closed"—"That is better"—"Continue breathing"—"In—out"—"Do not hurry"—"Do not work"—"Breathe just as you would through the nose" . . . etc.

It is well as far as possible to time such softly spoken admonitions to the rhythm of his breathing so that the necessary message is conveyed to him without jarring upon his nervous system as a shock from an unexpected noise, and his own natural breathing rate suggested to him by the rhythm of the admonition.

After starting the mouth breathing nothing at all is said unless necessary; the patient is not touched nor disturbed in any way; the pulse not taken during the test, but before and after. The only time it seems legitimate to touch a patient during a test is to lay an assuring hand upon a child who needs physical contact to quiet him and to help him over his momentary panic. Occasionally an exception is made to this rule in the case of an ex-

tremely nervous adult who needs assurance that all is well. Taking the pulse during the test is apt to carry with it a sinister suggestion that perhaps all is not well and that thereby his condition is being watched.

Plenty of time is spent in the process of instruction. Each patient not only is told to keep his lips closed around the rubber mouth piece but is instructed why. If he exhibits enough interest in the procedure to ask questions, his questions are intelligently answered. Often just such a series of questions and answers is what he needs to take him off of his tension. He becomes interested in the fact that he has learned something new and is anxious to try it out successfully.

Basal metabolism success is determined by many factors, among which psychology plays a very important part. You may not hurry your patient, nor appear to be hurried yourself. You must give him the impression that you have plenty of time, that you are both setting about the task in hand to do it correctly, not to get it done quickly.

If you walk into the basal metabolism room slowly, assuming a quiet, unhurried mien, you impart to the patient as if by magic the proper mood or psychology. You must not be afraid of him, be confident that you are going to conquer him regardless of how difficult he may prove to be. He will succeed if you compel him to; if you are afraid of him, you predetermine failure for him.

It is only in cases where the technical and mechanical factors have not reached the highest degree of perfection and balance that the interpretative power of the physician must sit in judgment.

By interpretative aspect we refer to that keen power of graph censorship whereby one is enabled to accept or reject as unsatisfactory the kymograph tracing submitted with its minus or plus value as indicated. There are other interpretative potentialities inherent in certain tracings indicative of existing blood chemical changes or properties, but a discussion of these falls outside the scope of the present undertaking. We shall limit our discussion to illustrations of tracings which record mechanical and technical perfection and imperfections, to differentiation of the acceptable

from the inacceptable, also to individual characteristics often seen in tracings which are not explicable as defects due to mechanics or technique.

The following illustrations, excepting charts 1 and 15, represent results obtained using the technique herein suggested.

## SUMMARY

In conclusion we enumerate the advantages resulting from the application of the technique described; tests repeated on normal individuals from year to year check within two or three per cent; the element of doubt as to the reliability of the estimation is almost completely removed because the procedure provides a prolonged stretch of kymograph tracing which usually is fairly free from evidence of unsatisfactory recording due to excited breath excursions; the prolonged time element is conducive to regularity of natural breathing; the psychology of the procedure is constructive and salubrious; the necessity of repeated tests because of unsatisfactory results arises very seldom; and the prolonged graphing falling upon or determining a consistent slope throughout its entire length provides two tests giving exactly the same result. When we keep in mind the fact that an initial test in certain cases is apt to run about five per cent higher than subsequent tests on the same individual under the same circumstances, no difficulty of judgment regarding the reliability of the result should arise.

Only by constantly respecting the three underlying factors predetermining success, mechanics, technique, and the knowledge necessary to intelligent graph censorship, is one able to produce outstanding results in this extremely fascinating field.

Success is very easy; failure, very "hard."

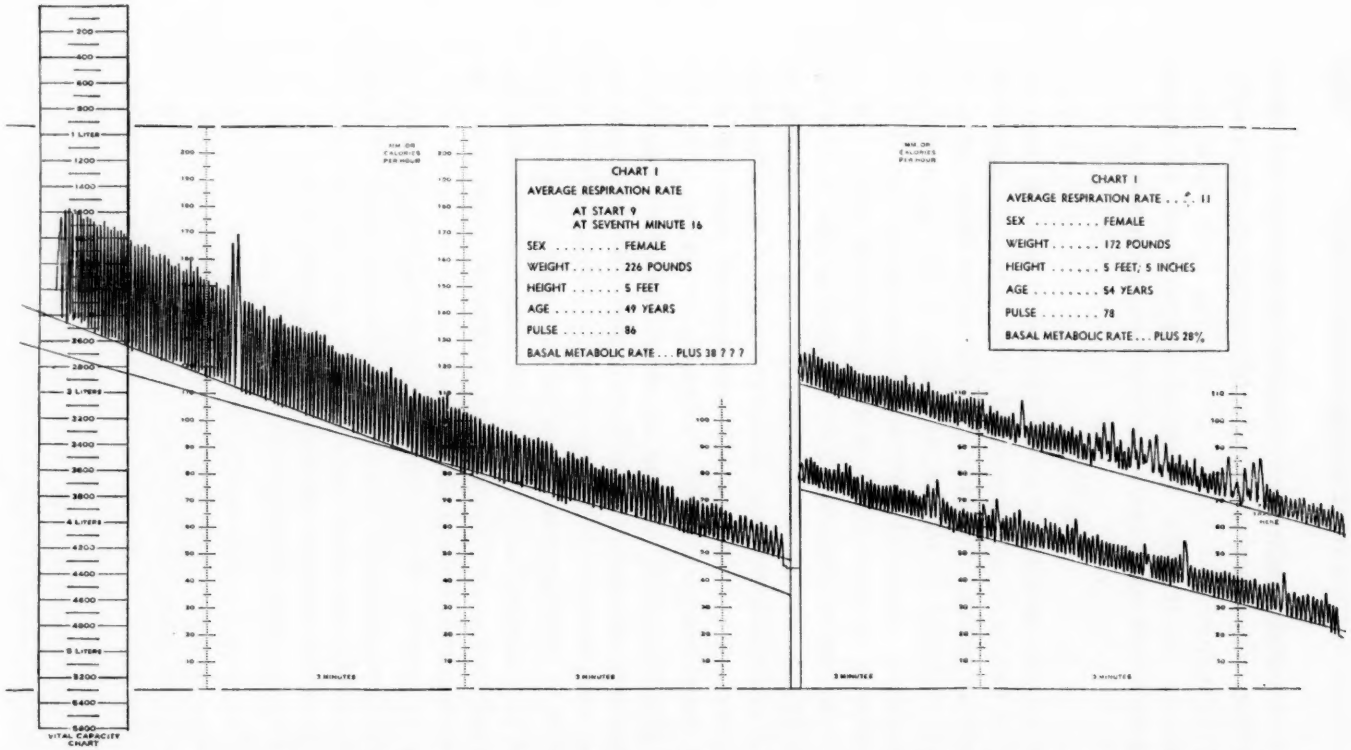



CHART 1. Represents two different tracings obtained out of our department; the first referred in duplicate for graph censorship records an obviously erroneous result. It reveals a progressively increasing respiration rate accompanied by a progressive lengthening of each successive breath. Across this graph we can write only one verdict, "Exhausted lime supply." The tracing on the right taken after providing a fresh lime supply confirms the validity of the recommendation. Aside from the general appearance and the impossibility of determining the average respiration rate, the result itself when viewed in the light of other factors, extreme weight, and normal pulse rate, does not merit credence.

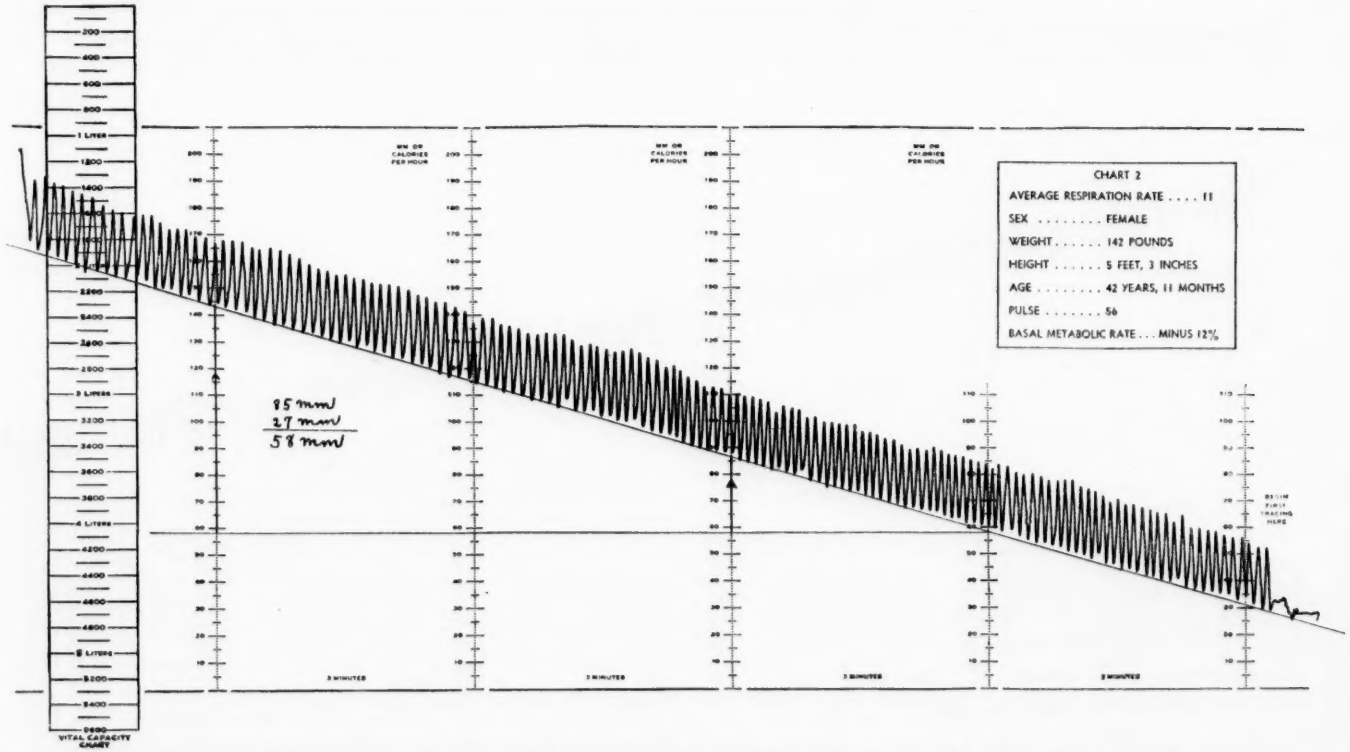



CHART 2. Represents as perfect a kymograph tracing as it is possible to obtain, regular and satisfactory in every way, demonstrating the fact that it is possible for a patient to breathe for a long or a prolonged time without interruption and with no difficulty to produce a perfect result.

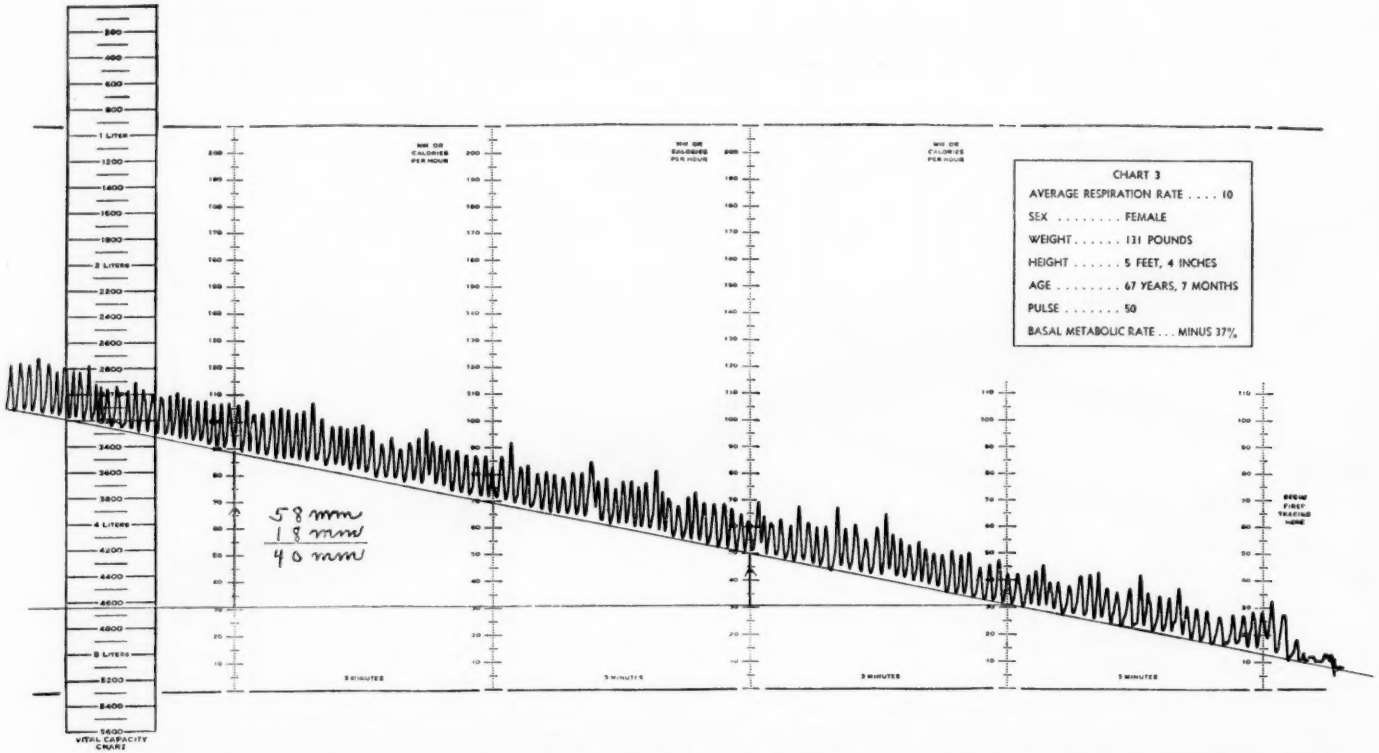



CHART 3. Illustrates the fact that the slightly irregular type of graph is the rule rather than the exception. The low respiration rate is responsible for the individual deflections falling rather far apart and the extremely low basal rate plus the incidence of slight stature determine the shallowness or incline of the slope.

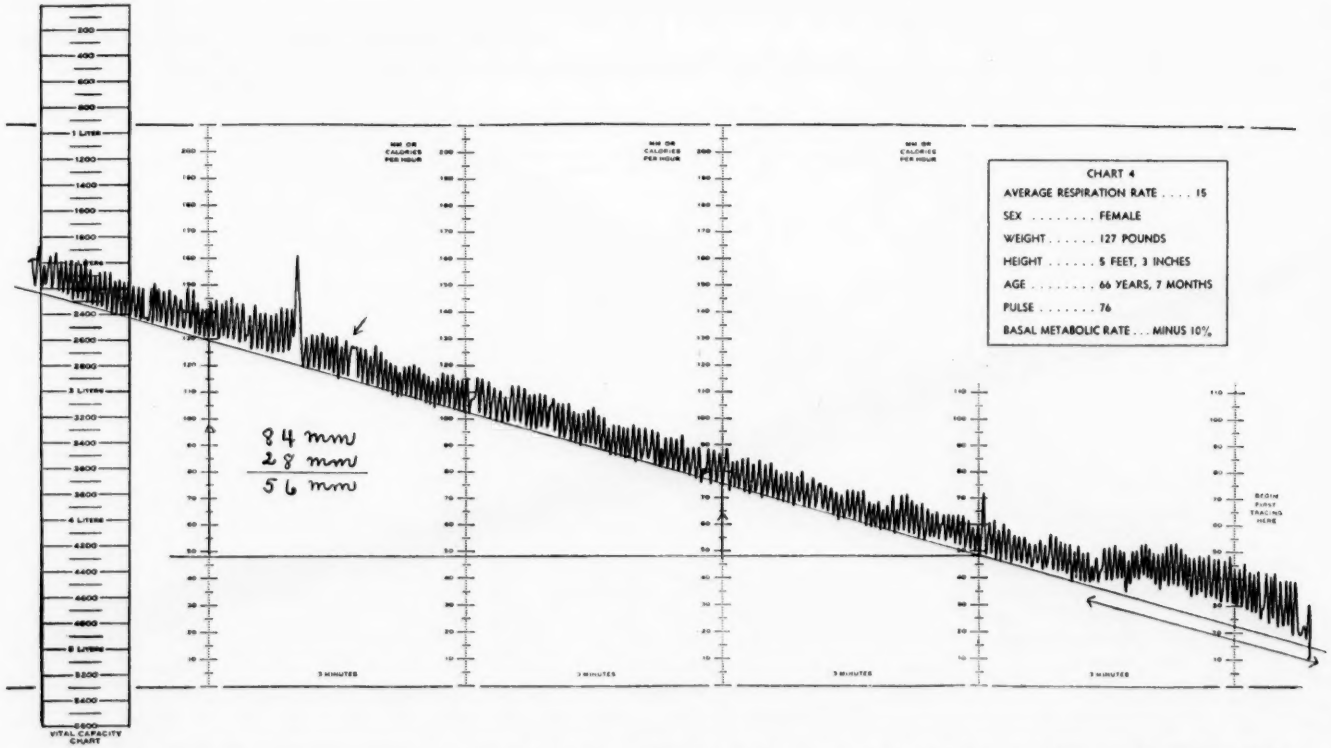



CHART 4. Records a discrepancy of slope falling throughout the duration of the first two minutes; these are disregarded as the basal rate does not become established until the third minute. The two long breaths are not defects but means of relief from the slight tedium of mouth breathing. The rather broadened breath deflection denoted by the arrow is due to a cessation or holding of the breath for a few seconds probably in an effort to swallow collecting saliva.

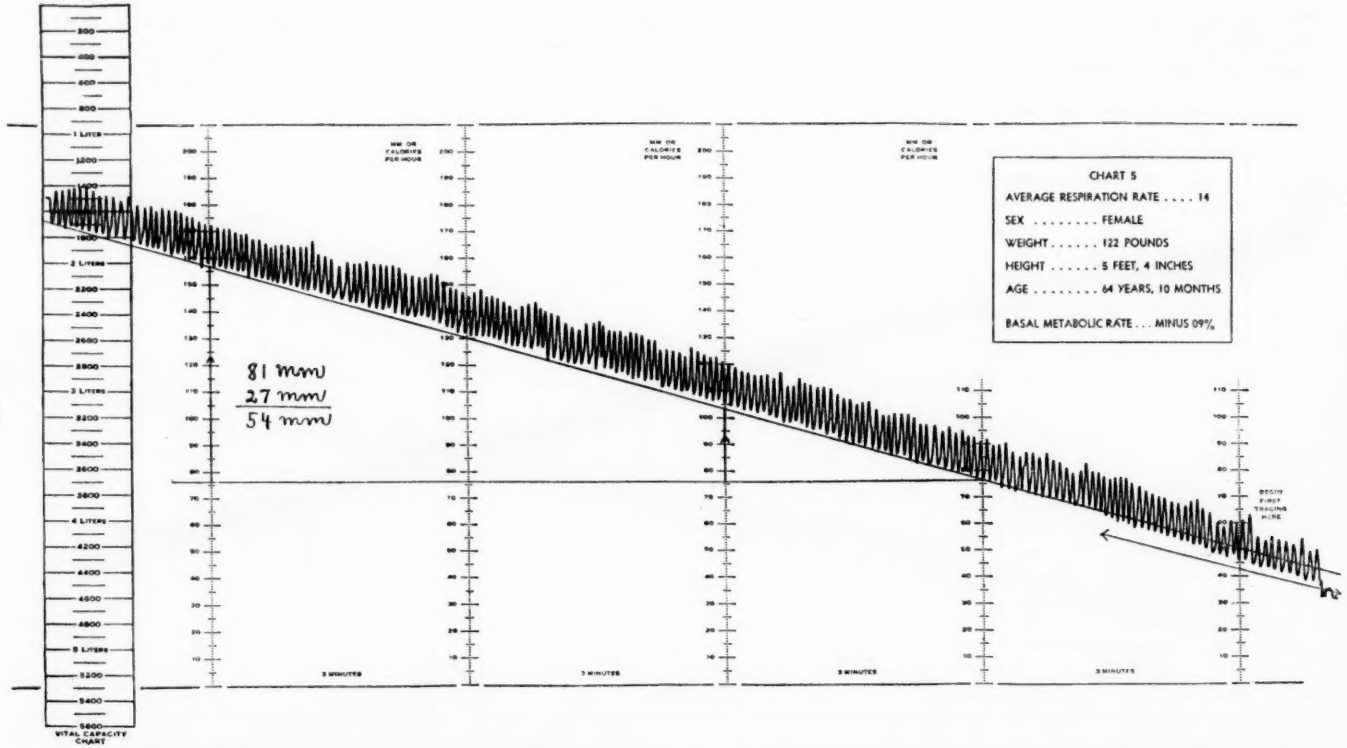



CHART 5. Reveals a new type of lineless chart which is replacing the older lined form. The kymograph recording aside from the first three minutes is extremely well done.

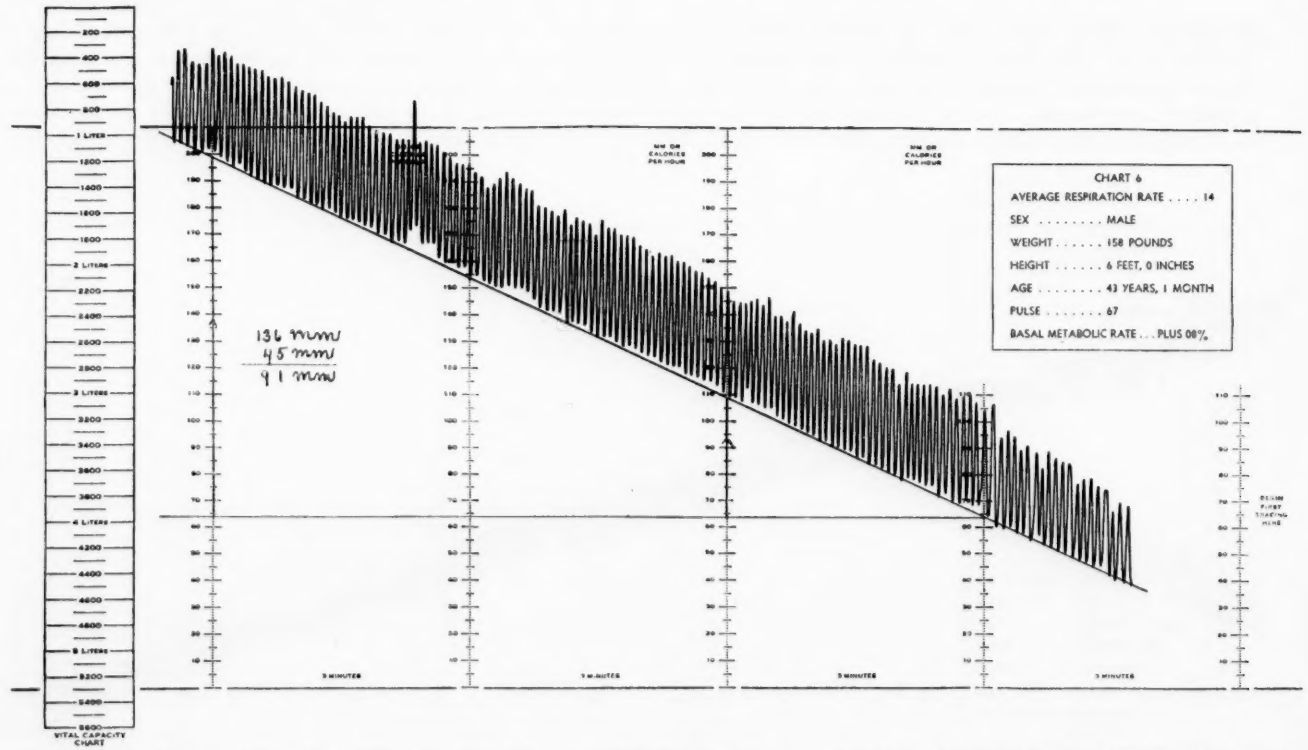



CHART 6. Demonstrates the inadequacy of a chart any smaller than the one used. If we had started the tracing lower on the paper we could have obtained two minutes more of graph; but it is not a good practice to fill the oxygen bell to capacity as it is inclined to tip or topple when its lower circumference is forced up to the surface of the water jacket, whereby exists a possibility of oxygen loss. The patient in this case, a male of considerable stature, without registering an advanced metabolic rate exhausts in only eleven minutes not only the chart but also the oxygen supply. If the chart were any smaller it would hazard any dependence we might place in the particular estimation.

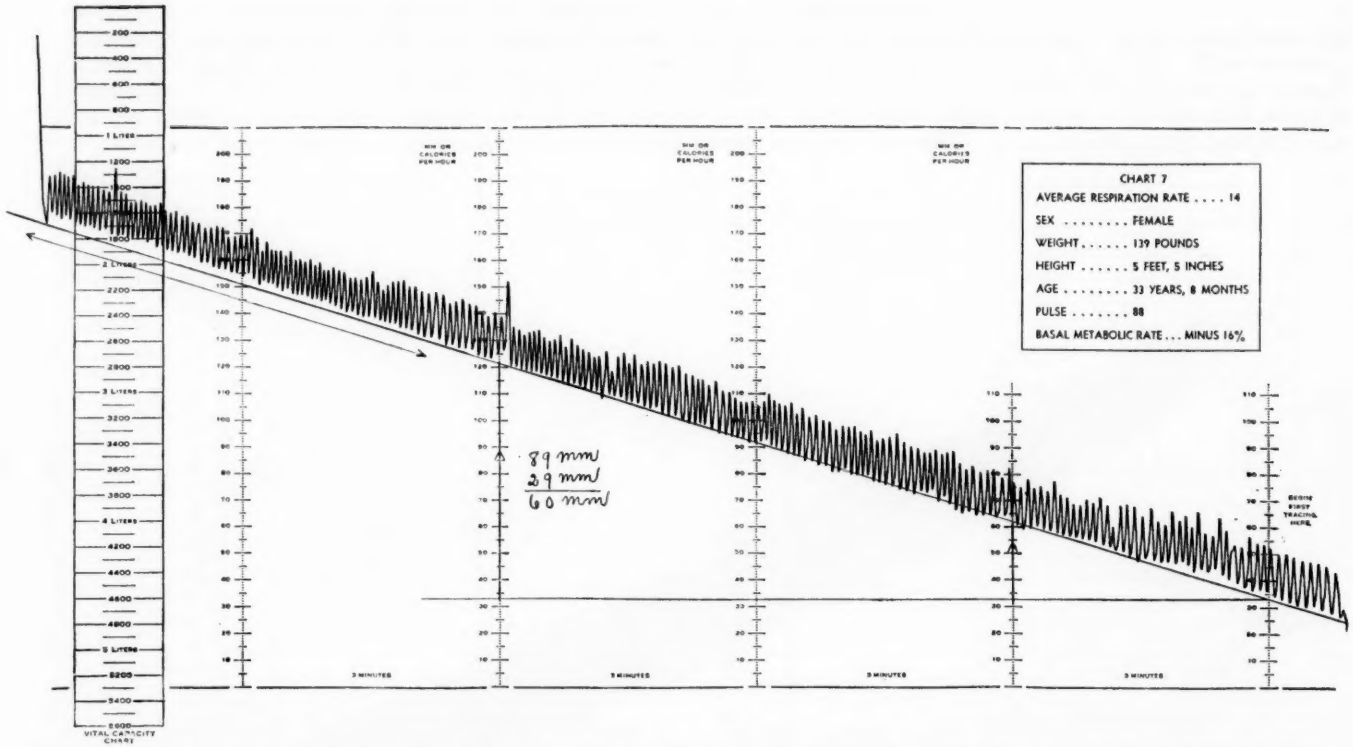
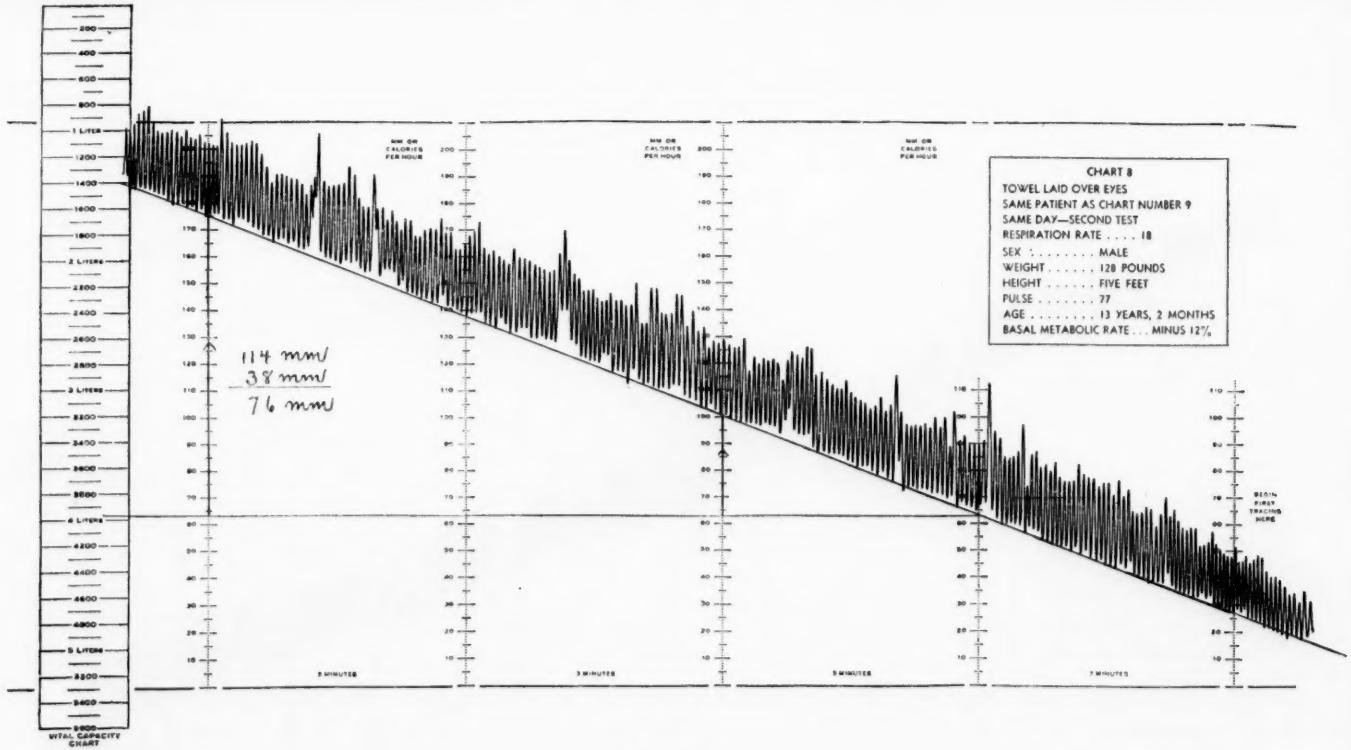



CHART 7. Illustrates a discrepancy of slope continuing throughout the last four minutes, due to the establishing of a leak so slight that it was not detected by noting a change in graph slope as the test progressed. Such a leak is usually due to a relaxing of the lips around the mouth piece allowing it to slip or become displaced. In such a case this section of graph is disregarded as we have already obtained ten minutes of satisfactory kymograph recording. It is possible that the preceding long breath initiated the muscle relaxation which made possible the leak. This graph throughout the first ten minutes records a reliable result.




## Chart 8

Charts 8 and 9, both taken on the same day, record the results of the first test of a boy of thirteen years. Throughout the first attempt, chart 9, in spite of repeated admonitions, the patient, mostly because of curiosity, persisted in opening his eyes at somewhat regular intervals. Because of this failure to cooperate completely we required him to make a second recording and fortified ourselves against repetition of the difficulty by laying a folded towel over the eyelids. We thereby were able to eliminate the long breaths from the second graph; the test satisfactorily repeated as shown by chart 8 registers a result three per cent lower than the original.

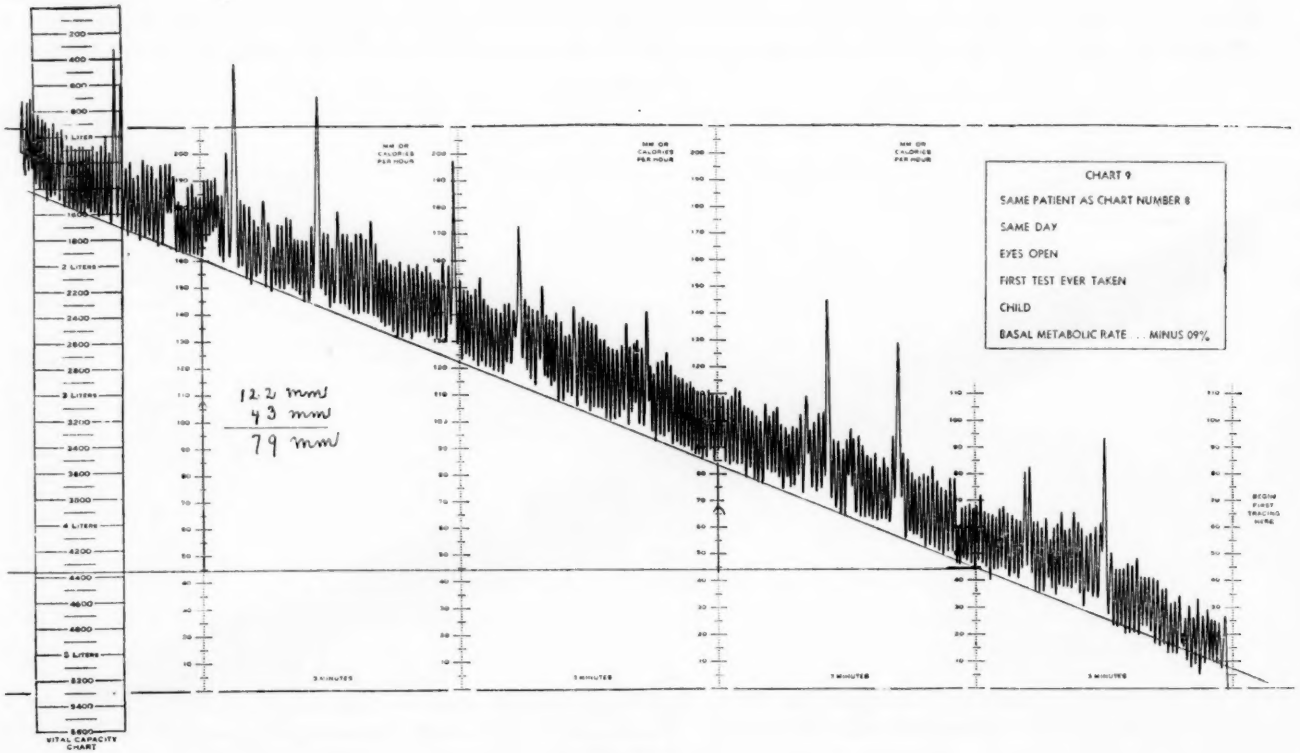


CHART 9
SAME PATIENT AS CHART NUMBER 8
SAME DAY
EYES OPEN
FIRST TEST EVER TAKEN
CHILD
BASAL METABOLIC RATE . . . MINUS 09%
12.2 mm
4.3 mm
7.9 mm
VITAL CAPACITY CHART
BEGIN FIRST TRACING HERE

CHART 9

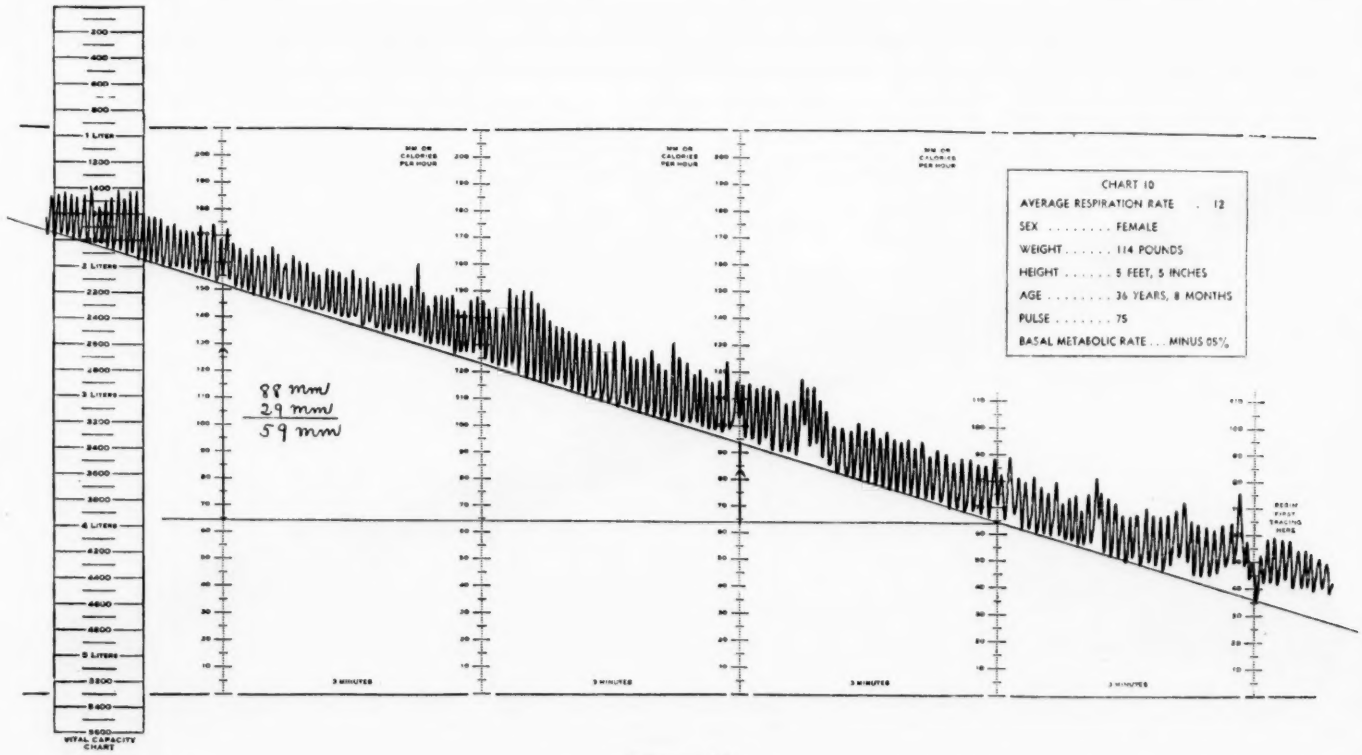



CHART 10

Charts 10 and 12 record slight pauses occurring along the exhalatory slope. It is this type of characteristic that the more recent investigators are designating as indicative of changes from the normal in certain blood chemical properties. The field is too new to attempt explanation beyond mere mention. Number ten tracing presents failure of the graph proper at two or three points to drop to the slope line. As it eventually returns to its self-determining hypotenuse, these few points of failure to conform are not to be considered defects but rather unavoidable irregularities. Here again we note the necessity of disregarding the first two minutes as exemplifying slope discrepancy.

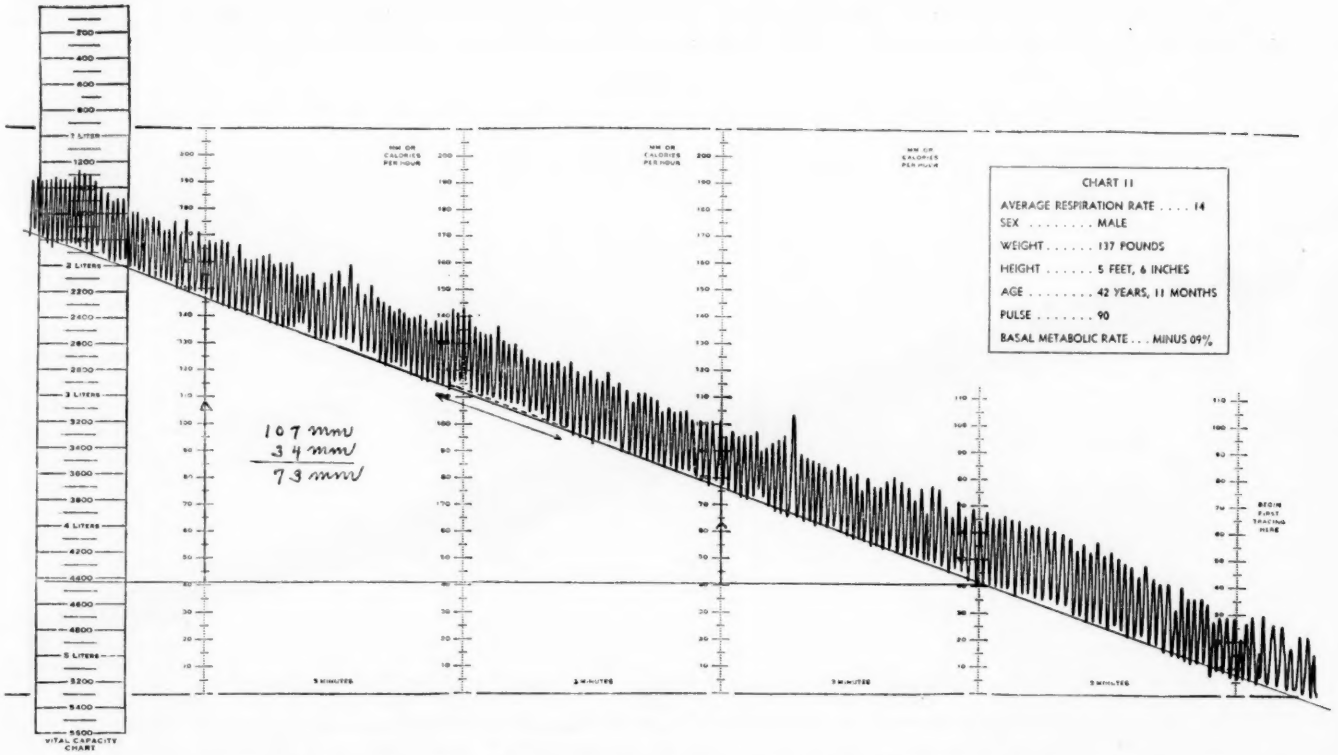



CHART 11. As indicated by the arrow points illustrates a slight rise of the graph from the slope line throughout the section recorded during the application of the leak tester. Upon removal the graph returns to the slope.

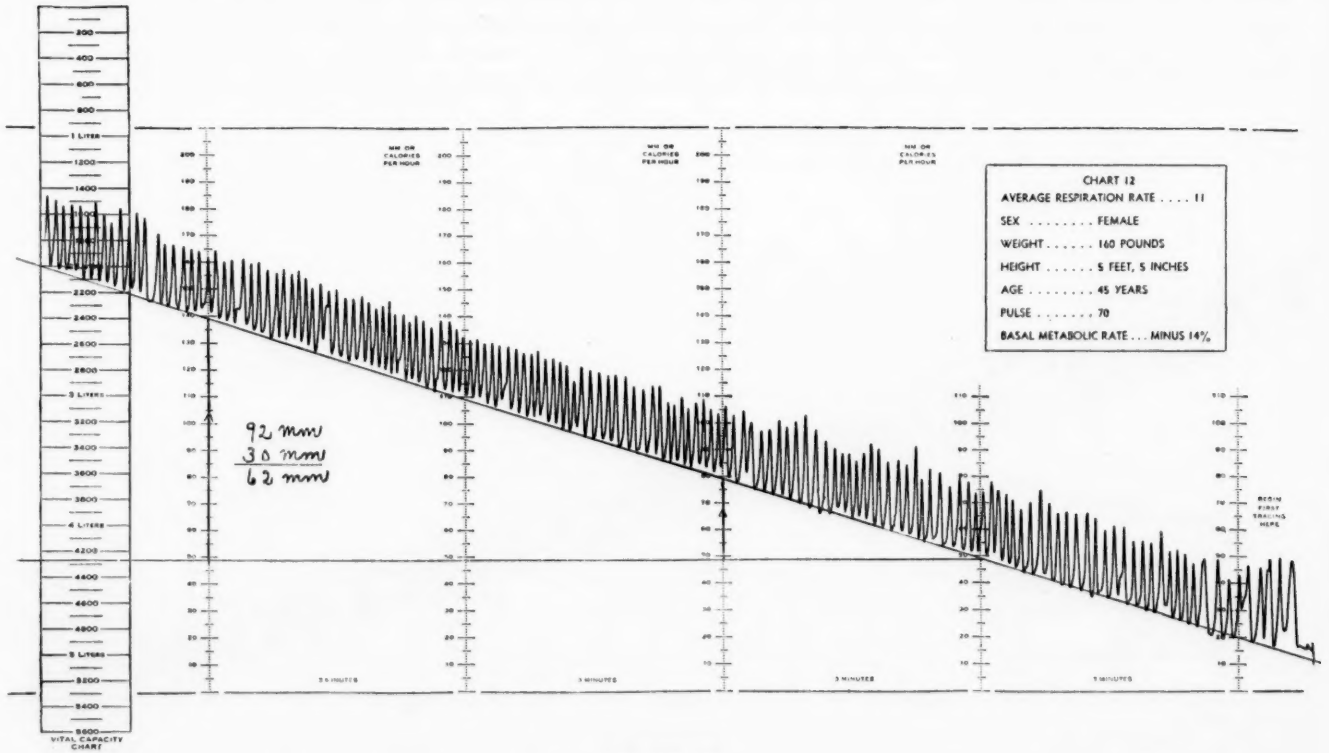


CHART 12
AVERAGE RESPIRATION RATE . . . . 11
SEX . . . . . FEMALE
WEIGHT . . . . . 160 POUNDS
HEIGHT . . . . . 5 FEET, 5 INCHES
AGE . . . . . 45 YEARS
PULSE . . . . . 70
BASAL METABOLIC RATE . . . MINUS 14%
92 mm
30 mm
62 mm
BEGIN FIRST TRACING HERE
VITAL CAPACITY CHART

CHART 12

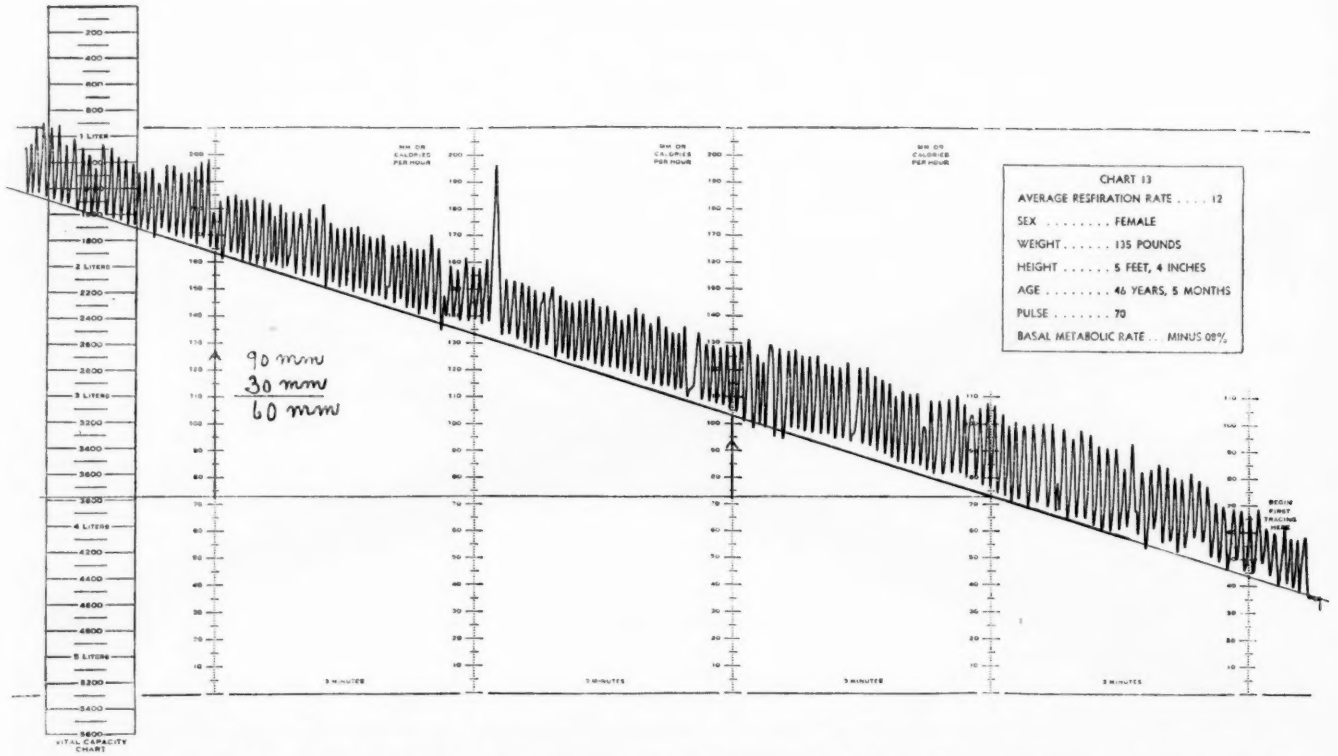



CHART 13. Records one very long deflection representing the distance traveled by the pen during a very long excursion of the breath. This is not a defect, but rather a point of relief rendering the remainder of the tracing easier than it would otherwise have been. Also certain downstrokes representing exhalations reveal a break in their integrity or unity. These points of hesitation in the progress of the breath are to be considered means of relief from the strain rather than technical or mechanical defects.

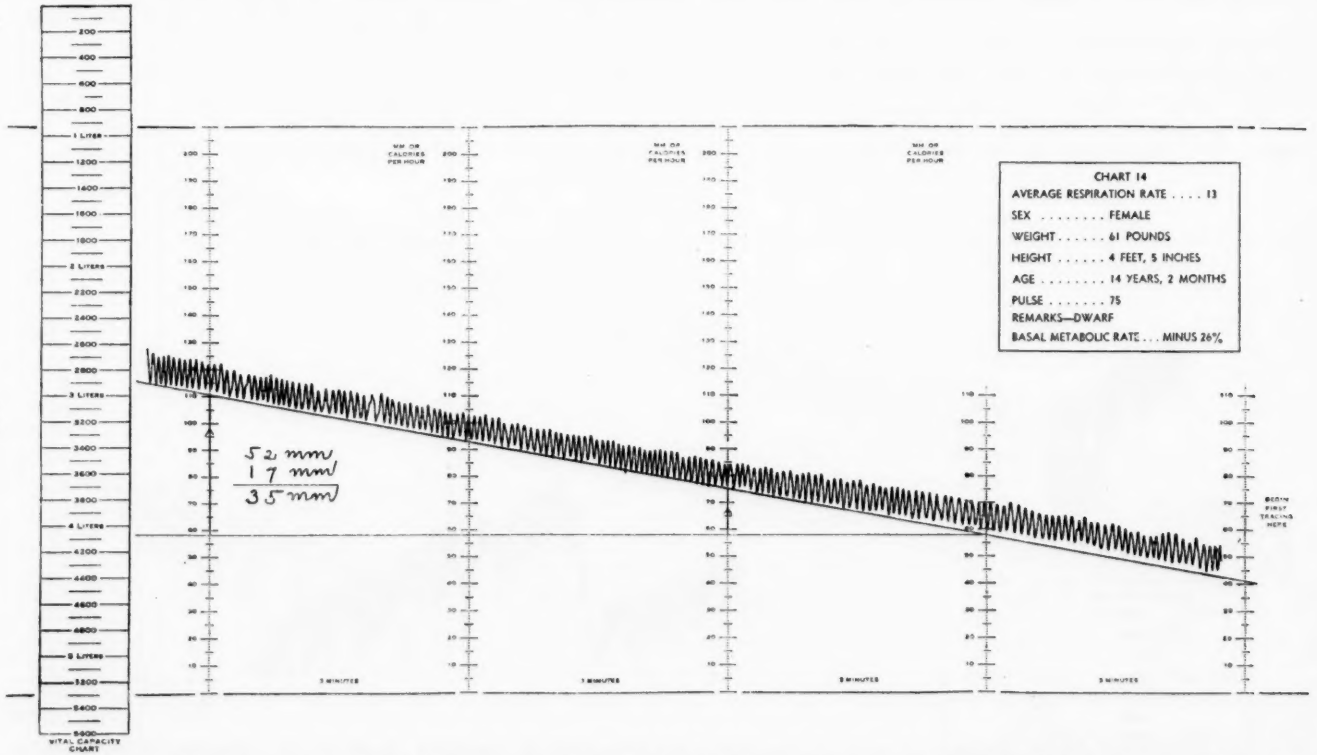



CHART 14. Records a perfect graph effect traced by a child of fourteen years, a dwarf, weighing sixty-one pounds, stature four feet, five inches, mentality not over average. The diminutive size plus the low basal rate accounts for the extreme shallowness of slope. The first three minutes as usual illustrate legitimate slope discrepancy.

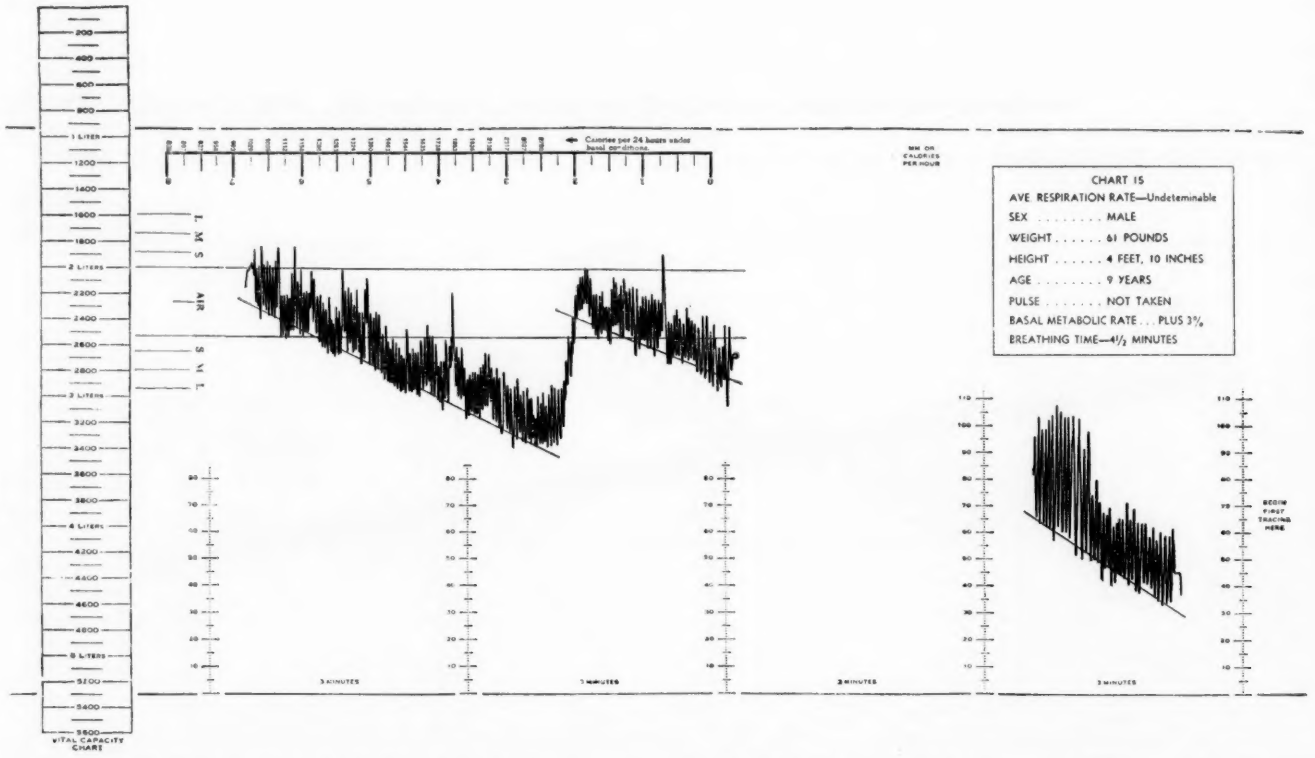



CHART 15. Represents a chart of inadequate size as compared with the larger one which serves as a background. Even the larger one is none too large. The graph in the lower right corner recorded upon the diminutive chart is typical of that obtained from the panic-stricken patient; he hurries, works, breathes irregularly, does everything we instruct him not to do and in addition is limited to four and one-half minutes for no other reason than that the unscientific design of the machine does not provide a chart large enough to allow him to breathe a long time so that he may establish his basal rate after he has overcome the first few minutes of terror.

Also the short stretch at the left illustrates unsatisfactory recording, the patient's equilibrium not having become established.

Also the short stretch at the left illustrates unsatisfactory recording, the patient's equilibrium not having become established.

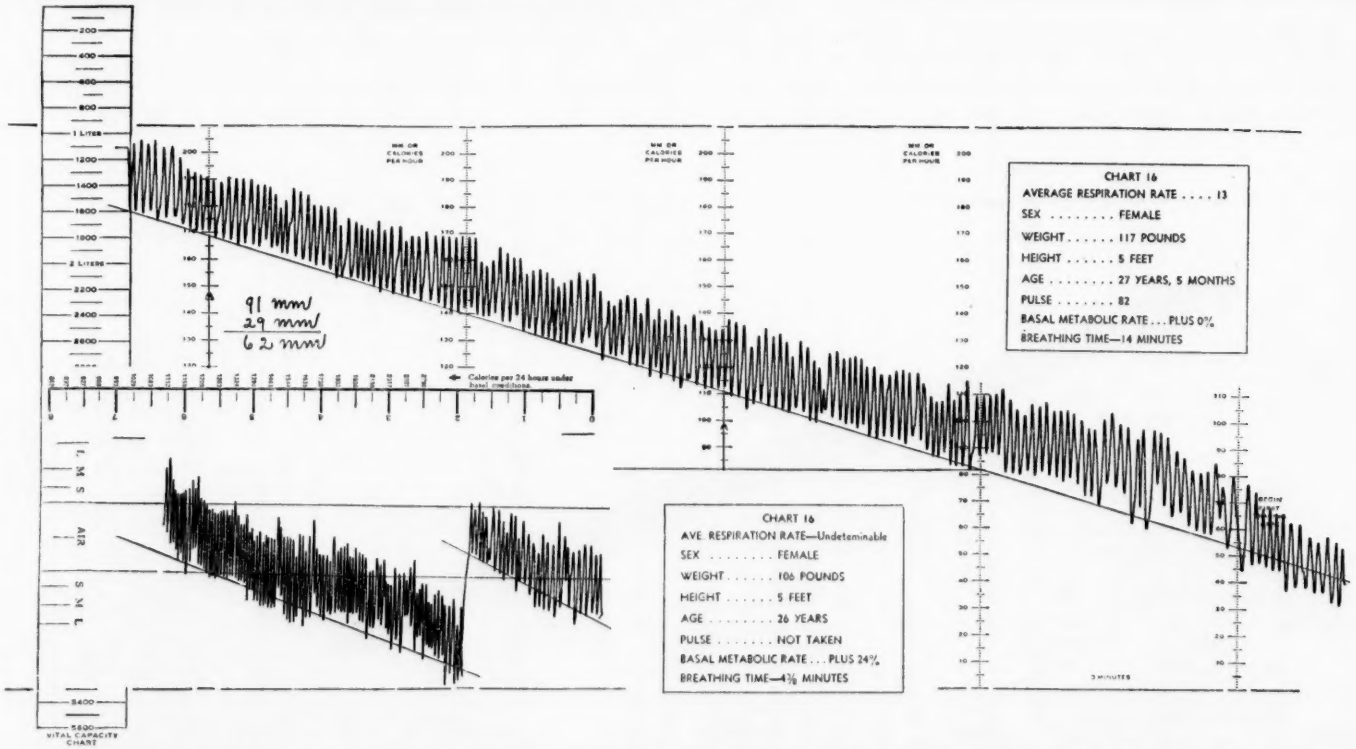



CHART 16. Emphasizes the necessity for large charts and prolonged breathing time in order to obtain reliable results. The two tracings were made by the same patient in the hands of different operators. Again the small chart limits the breathing time to four and three-eights minutes while the larger one provides every possibility for recording a reliable result. The basal metabolic rates as represented by the limited technique is plus 24 as compared to plus 0 as calculated from the large record. This patient was referred by a certain roentgenologist who was unwilling to proceed with treatment until he had obtained a credible result. It is almost unbelievable that the result in the lower corner was submitted from a goitre clinic of some note.

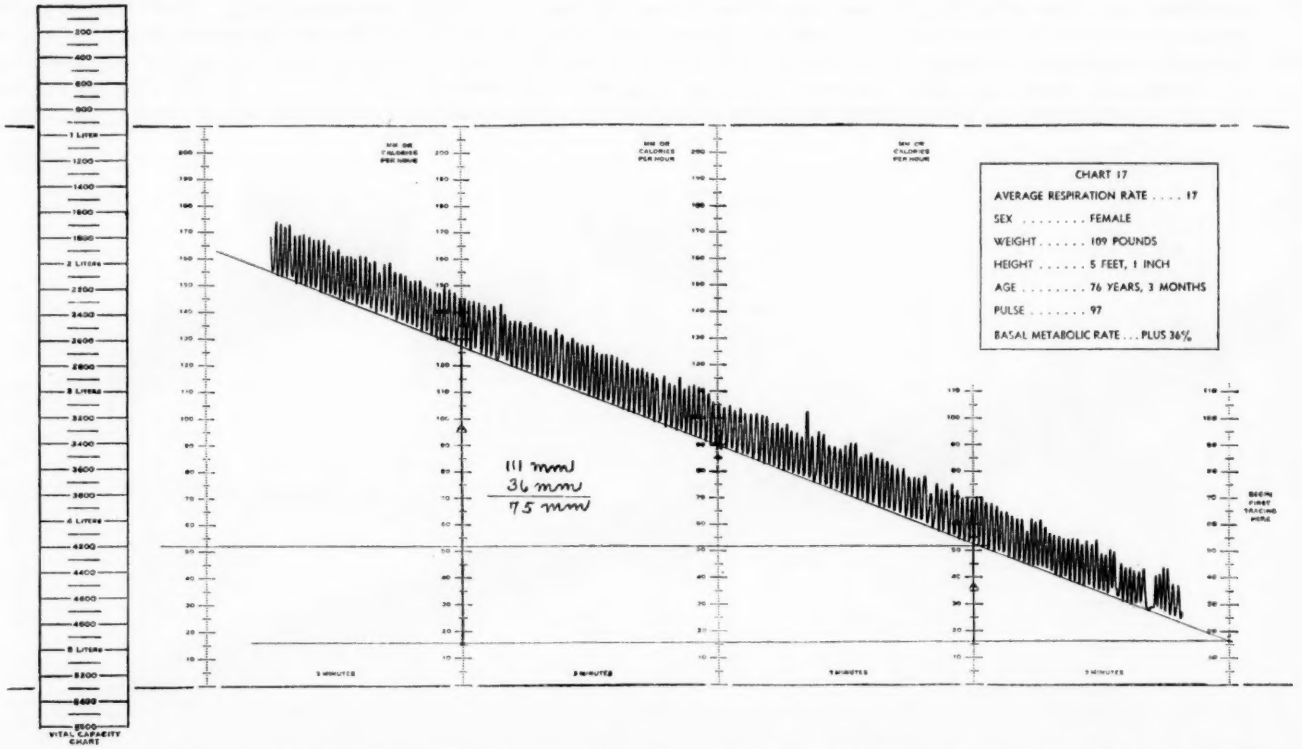



CHART 17. Illustrates the possibility of perfect prolonged recording even in the case of a very elderly feeble hyperthyroid of the toxic adenoma type whose age was seventy-six years, whose basal metabolic rate proved to be plus thirty-six. We did not require her to complete the last four minutes as we were obliged to steady her head as she breathed in order to keep her from shaking loose from the mouth piece and rubber tubing, as she presented a very marked tremor of head and body almost as pronounced as a Parkinsonian tremor.

# A MODIFICATION OF THE KRAMER AND TISDALL METHOD FOR DETERMINING POTASSIUM IN SERUM*

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In 1933 one of the authors published a note upon a technic for precipitating potassium as potassium sodium cobaltinitrite[1]. This technic regularly yielded a precipitate of constant composition which corresponded with one of the theoretical formulae of such compounds. A method for the determination of potassium in serum based upon these experiments has been developed, and has been applied successfully by a number of technicians, both in this clinic and in various other institutions, during the last four years. The method appears to possess certain advantages over some of the others in use[2], and these seem to make it worth while describing the procedure in detail.

*Reagents:* (1) Sodium acetate. 100 grams of pure crystalline salt dissolved in a total volume of 250 cc. with distilled water. (2) A solution of sodium cobaltinitrite prepared according to the directions of Kramer and Tisdall[3]. Dissolve 25 mgm. cobaltinitrate in 50 cc. of distilled water, add 125 cc. glacial acetic acid to give solution "A." Prepare solution "B" by dissolving 120 grams of sodium nitrite free from potassium in 180 cc. of distilled water. Add 210 cc. of solution "B" to all of solution "A." Draw air through for about 2 hours to remove the free gas. The solution should be kept on ice and discarded if more than 3 months old. It should be filtered just before it is used. (3) Pure acetone. (4) 25 cc. of acetone diluted to 100 cc. with distilled water. (5) Pure concentrated nitric acid kept free from ammonia. (6) 2 per cent solution of pure anhydrous sodium phosphate. (7) Approximately 4 normal sulfuric acid made by adding 20 grams of the best grade concentrated acid to distilled water and diluting to 100 cc. (8) Fiftieth normal potassium permanganate freshly prepared from stock tenth normal solution containing 3.162 grams per liter. (9) Accurately prepared fiftieth normal sodium oxalate. This

* Received for publication July 18th, 1938.

is made by diluting a tenth normal solution, which in turn is made by dissolving 6.7 grams of Sørensen's salt in a liter of distilled water containing 5 cc. of pure concentrated sulphuric acid.

*Procedure:* Measure 1 cc. of serum into a 15 cc. centrifuge tube of pyrex glass. The material should be fresh, hemolysis should be absent, and the serum should be separated from the clot as soon as possible after the blood is drawn.* The potassium content should be between 5 and 80 mgm. per 100 cc. Add 1 cc. of sodium acetate, followed by 1 cc. of the mixed, filtered, Kramer and Tisdall reagent. No special precautions need be taken in adding this reagent. Mix and set the tube in a bath of ice water for between one-half and 2 hours. Centrifuge, decant, and drain on a towel. Wash with 5 cc. of the dilute acetone, mixing the acetone with the precipitate as thoroughly as possible. Again centrifuge, decant, and drain. Add 0.1 cc. of concentrated nitric acid and heat in a beaker of boiling water until the precipitate is dissolved and the supernatant liquid, which is at first cloudy, probably due to a trace of precipitated protein, is clear. This process usually requires from 5 to 10 minutes, but may take somewhat longer. Add again 1 cc. of sodium acetate solution, 1 cc. of the sodium cobaltinitrite reagent, and again set in ice water for half an hour or more. Centrifuge, decant and drain, and wash with 5 cc. of the diluted acetone. Decant and drain and wash at least once with pure acetone. It is important that the washing solutions should be thoroughly mixed with the precipitate. Centrifuge, decant, drain, and allow to dry after the last washing. Finish drying in a boiling water bath and remove the last traces of vapor present by suction. Add to the tube containing the dried precipitate 2 cc. of 2 per cent sodium phosphate and a clean glass rod. Stir thoroughly and heat on a boiling water bath until all the precipitate is decomposed and the cobalt is present as the insoluble phosphate. Bring to room temperature, add 1 cc. of 4 normal sulfuric acid, and titrate at once with fiftieth normal potassium permanganate. This may conveniently be carried out by adding rapidly, and with as little stirring as possible, a slight excess of permanganate from an accurately calibrated burette reading at least to one-fiftieth of a cc. Place the tube in a water bath at a temperature of about 60°C. and after 5 minutes add half a cc. of fiftieth normal sodium oxalate to give a colorless solution. If too great an excess of permanganate was added in the initial stage of the titration larger amounts of oxalate must be used. Carry out a back titration to the first permanent pink color, adding the permanganate from the same burette used in the first part of the procedure. Determine the blank given by half a cc. of oxalate, or, if a larger amount of oxalate was added, by the amount actually used in the presence of the amounts of phosphate and acid solutions employed

* The method has not as yet been successfully applied to plasma. The precipitate given by the Kramer and Tisdall reagent floats on the surface and cannot be separated by centrifuging. This difficulty seems to be caused by the presence of fibrinogen.

in dissolving and acidifying the potassium sodium cobaltinitrite precipitate. Standardize the potassium permanganate against the oxalate in the usual way.* The presence of dust should, of course, be carefully avoided throughout the procedure, and it has therefore been our custom to keep the centrifuge tubes covered with rubber caps whenever this was possible. All glassware must be scrupulously clean. The burette used in the final titration should be cleaned with chromic acid frequently.

Calculation: "A" = total cc. of permanganate used in both parts of the titration. "B" = cc. of permanganate measuring the blank due to the oxalate, phosphate and acid. "A" − "B" × factor of permanganate × 0.13 × 100 = mgm. potassium in 100 cc. of serum.

A large number of experiments were carried out in studying the accuracy of this method. Those dealing with the limitations upon the precipitation of potassium from pure solutions have been discussed in the preceding publication. In addition to the factors considered there the recovery of potassium added to serum was investigated. The table shows that this recovery was satisfactory. It was also shown that duplicate determinations made upon various aliquots of the same specimen of blood serum (1 cc. of serum compared with 1 cc. of the same serum diluted with an equal volume of sodium chloride, etc.) gave satisfactory agreement.

The double precipitation method was adopted because the precipitate obtained directly from serum is often not suitable for quantitative analysis as it is so closely matted together as to

* The following method of titrating is simpler than that described in the text, and seemed to give very good quantitative results, although the end-point is somewhat more difficult to duplicate. After decomposing the precipitate of potassium sodium cobaltinitrite with phosphate and heat and acidifying, titrate rapidly in the cold almost to the end-point. When the color fades, slowly immerse in a beaker of boiling water and finish the titration, taking as the end-point the first pink color which will persist for 10 seconds when the tube is returned to the boiling water bath. Standardize the permanganate to the same end-point in the same way, and determine the blank given by the reagents. The calculation is the same as that given in the text. The amount of nitrite lost in this procedure forms such a small per cent of the total amount present that the results check with the theoretical ones in a very satisfactory manner.

make thorough washing impracticable. It seems, too, to contain impurities which are oxidized by permanganate[4]. The authors have therefore resorted to the method of purification described which resembles that proposed by Fiske and Litarczek[5]. Not only was recovery satisfactory when 2 or more precipitations were carried out, but the method recommended gave identical results with those obtained after alkaline fusion of the first precipitate with sodium carbonate—a method which removes very large amounts of organic impurities and ammonia and permits quantitative recovery of potassium.

The method of purification with nitric acid is not applicable when significant amounts of ammonia are present. It can be

TABLE 1

| EXTRA POTASSIUM ADDED TO 1 CC. OF SERUM* | EXTRA POTASSIUM FOUND IN 1 CC. OF SERUM† | | |
|---|---|---|---|
| *mgm.* | *mgm.* | | |
| 0.025 | 0.022 | 0.025 | 0.021 |
| 0.05 | 0.050 | 0.055 | 0.047 |
| 0.10 | 0.101 | 0.102 | |
| 0.20 | 0.196 | 0.201 | 0.200 |
| 0.30 | 0.297 | 0.298 | 0.285 |

* Potassium added as pure potassium sulfate solution.

† Corrected for the potassium content of the serum.

used in the analysis of serum because that compound is either absent or present in such low concentrations as not to be precipitated. A fairly convenient method was developed for use when, as in urine, ammonia is present, but other interfering compounds such as protein are absent or present only in traces. The urine was first diluted one part to ten with distilled water. One cc. of this material was precipitated and washed as first described. The precipitate first obtained was then treated with an excess of sodium bicarbonate, and the test tube immersed in a boiling bath containing 25 to 30 per cent sodium chloride, and the contents evaporated to dryness. During the evaporation a current of air was drawn through the solution to prevent mechanical loss of material through "bumping." This method was tested out on

a large series of urine specimens and found to give satisfactory results.

SUMMARY

A modification of Kramer and Tisdall's method for determining potassium in serum is described. The method is applicable to concentrations of potassium between 5 and 80 mgm. of potassium per 100 cc. of serum, and by diluting the serum with salt solution can be applied when any higher concentration is present.

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## A LINEAR RELATION FOR CALCULATING BLOOD SUGARS BY THE TIMED FERRICYANIDE METHOD*

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The timed ferricyanide reduction method of Hawkins and Van Slyke[1,2] is convenient, because it furnishes a rapid and sufficiently accurate determination without expensive equipment.

* Received for publication, June 28th, 1938.

The method, briefly, consists in heating an alkaline solution of potassium ferricyanide with an equal volume of 1:10 or 1:11 Folin-Wu filtrate and noting the time required for the yellow color of the ferricyanide to be bleached out of the solution. The sugar concentration in the blood is then read from an empirical curve given by the authors or determined by calibration with standard sugar solutions.

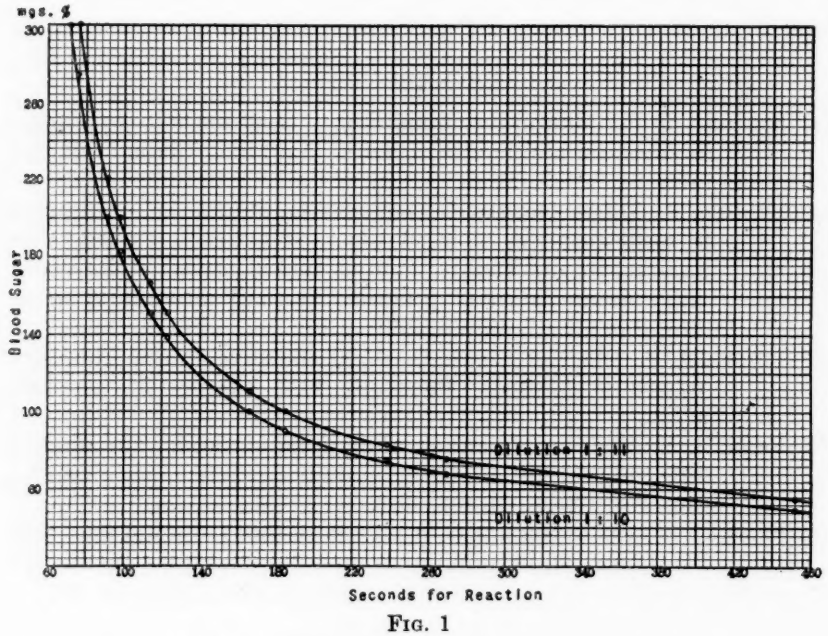


FIG. 1

The curve (fig. 1) takes the form of a negative exponential function. This is rather difficult to reproduce. However, if we plot the *logarithm* of the sugar concentration against the *reciprocal* of the time, the points fall on a straight line within the error of the method. This is conveniently done on semilogarithmic paper, plotting the blood sugars as ordinates and the reciprocals of the times as abscissae (fig. 2). The straight line is purely fortuitous. There is apparently no readily deducible theoretical basis for such a relation.

The times determined by Hawkins and Van Slyke for varying sugar concentrations are given in the following tables:

*1:11 Blood Filtrate*

| | | | | | | |
|---|---|---|---|---|---|---|
| Blood Sugar..... | 300 | 200 | 150 | 100 | 75 | 50 mgm. per 100 cc. |
| Time..... | 76 | 98 | 123 | 184 | 269 | 520 seconds |

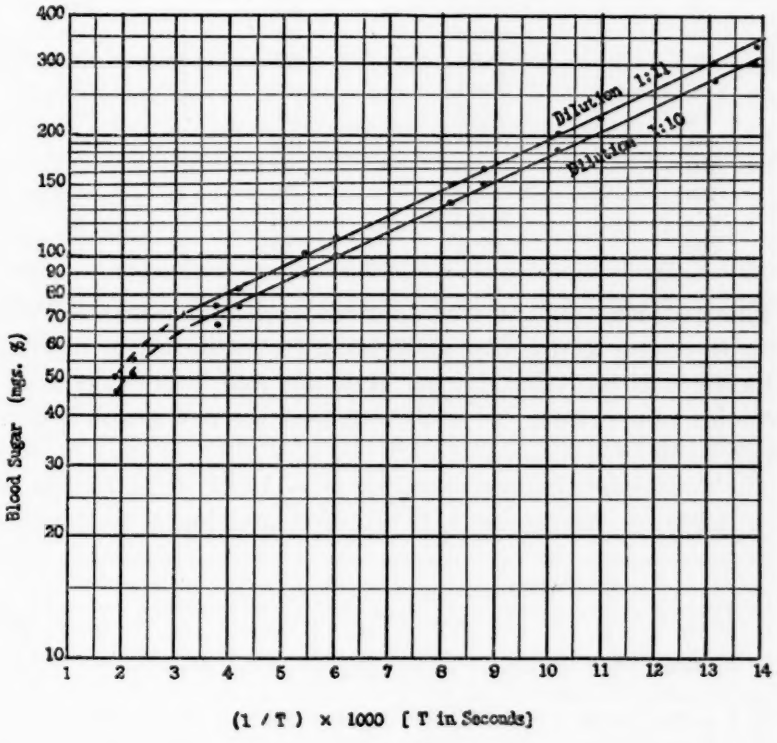


FIG. 2

*1:10 Blood Filtrate*

| | | | | | | |
|---|---|---|---|---|---|---|
| Blood Sugar..... | 300 | 200 | 150 | 100 | 75 | 50 mgm. per 100 cc. |
| Time..... | 71 | 91 | 114 | 166 | 238 | 451 seconds |

Each of the above sets of data may be used to supplement the other. For example, the 300 blood sugar with the 1:10 dilution is equivalent to a 330 blood sugar diluted 1:11, etc. We can

therefore obtain twice as many points for each curve, as follows:

| TIME | $1/t$ | (1:11) SUGAR | (1:10) SUGAR |
|---|---|---|---|
| 71 | 0.01408 | 330 | 300 |
| 76 | 0.01315 | 300 | 273 |
| 91 | 0.01099 | 220 | 200 |
| 98 | 0.01020 | 200 | 182 |
| 114 | 0.00878 | 165 | 150 |
| 123 | 0.00813 | 150 | 136 |
| 166 | 0.00602 | 110 | 100 |
| 184 | 0.00544 | 100 | 91 |
| 238 | 0.00420 | 83 | 75 |
| 269 | 0.00372 | 75 | 68 |
| 451 | 0.00222 | 55 | 50 |
| 520 | 0.00192 | 50 | 45 |

These values, when plotted on semilogarithmic paper, fall very close to a pair of parallel straight lines, as shown in figure 2.

Figure 1 is reproduced from Hawkins and Van Slyke and is given for comparison. It is obvious that the new graph is much easier to draw.

The equations of these lines, $\log S = 1.641 + 64.75/t$ or $\log S = 1.641 + 259/4t$ for 1:11 dilution and $\log S = 1.599 +$

| 1:11 DILUTION | | | | | | 1:10 DILUTION | | | |
|---|---|---|---|---|---|---|---|---|---|
| A | B] $259/4t$ | C $\log S$ | D $S$ (calc.) | E $S$ | F Per cent error | C′ $\log S$ | D′ $S$ (calc.) | E′ $S$ | F′ Per cent error |
| 71 | 0.912 | 2.553 | 357 | 330 | +8 | 2.511 | 324 | 300 | +8 |
| 76 | 0.852 | 2.493 | 310 | 300 | +3 | 2.451 | 282 | 273 | +3 |
| 91 | 0.712 | 2.353 | 225 | 220 | +2 | 2.311 | 205 | 200 | +2 |
| 98 | 0.661 | 2.302 | 200 | 200 | 0 | 2.260 | 182 | 182 | −0 |
| 114 | 0.568 | 2.209 | 162 | 165 | −2 | 2.167 | 147 | 150 | −2 |
| 123 | 0.527 | 2.168 | 147 | 150 | −2 | 2.126 | 134 | 136 | −2 |
| 166 | 0.390 | 2.031 | 107 | 110 | −3 | 1.989 | 97 | 100 | −3 |
| 184 | 0.352 | 1.993 | 98 | 100 | −2 | 1.951 | 89 | 91 | −2 |
| 238 | 0.272 | 1.913 | 82 | 83 | −1 | 1.871 | 74 | 75 | −1 |
| 269 | 0.241 | 1.882 | 76 | 75 | +1 | 1.840 | 69 | 68 | +1 |
| 451 | 0.143 | 1.784 | 61 | 55 | +10 | 1.742 | 55 | 50 | +10 |
| 520 | 0.124 | 1.765 | 58 | 50 | +16 | 1.723 | 53 | 45 | +17 |

$64.75/t$ or $\log S = 1.599 + 259/4t$ for 1:10 dilution can be used with a 10 inch slide rule to calculate the values of $S$. The table at the bottom of page 126 shows the accuracy of slide rule calculation.

The figures in column C are obtained by adding 1.641 to those in column B. Column D is gotten by taking the antilogarithm of column C. The figures of column C′ equal those of column B + 1.599 and D′ contains the antilogarithms of column C′. Columns E and E′ contain the data of Hawkins and Van Slyke.

These figures show that the relation is valid within the accuracy of the method for blood sugars from about 70 to 300.

The new graph lends itself admirably to the calibration of the method with newly prepared solutions. Dilutions of standard sugar solutions corresponding to concentrations of 100 and 250 mgm. per 100 cc. are prepared and timed. The average of several determinations is then used either to plot the relation or to calculate the constants of the equation,

$$\log S = a + b/t.$$

If $t_{100}$ and $t_{250}$ are the times for the two concentrations, then

$$\log 100 = a + b/t_{100}$$

and

$$\log 250 = a + b/t_{250}$$

Or

$$a + b/t_{100} = 2$$

$$a + b/t_{250} = 2.3979$$

Solving these equations, we obtain

$$a = 2 - 0.3979 \times t_{250} \div (t_{100} - t_{250})$$

$$b = 0.3979 \times t_{100} \times t_{250} \div (t_{100} - t_{250})$$

Either the graph or the equation can then be used for determinations with the solution thus calibrated.

The solution is prepared by dissolving 0.5 gram of purest potassium ferricyanide and 75 grams each of potassium carbonate

and bicarbonate in water and making up to a liter. Only the ferricyanide must be weighed out accurately.

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## AN ACCURATE PIPETTE CALIBRATOR AND MICROASPIRATOR*

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In standardizing pipettes for the Haskins-Sahli[1] and Osgood-Haskins hemoglobin methods[2], appreciable errors were found in pipettes commercially available, and small but significant errors even in those bearing the Bureau of Standards certificate. Calibration by weighing mercury was time-consuming where large numbers of pipettes were to be calibrated. The instrument herein described was, therefore, designed and constructed. It has been used with great satisfaction for several years. On looking up the literature, preparatory to describing the apparatus for publication, it was found that calibrators somewhat similar in principle have been described before[3]. However, the present instrument† has certain advantages which justify its description.

The principle of the method is the displacement of fluid by cylinders propelled by micrometers.

A stainless steel block, *A*, of suitable size is drilled as shown in figure 1. The block, *A*, in our instrument measures 1 x 1.25 x 5.5 inches. Two Brown and Sharpe (catalogue number 294) micrometers, *B* and *B'*, readable to 0.0001 of an inch are procured. Using a lathe, the small steel rod, *C*, is exactly centered and securely soldered to the steel plunger of *B'*. The holes, *D* and *E*, should be drilled to give as perfect a fit for the plunger of the micrometer, *B*, and the rod, *C*, as possible. The small rod, *C*, on the instrument we use is

* Received for publication August 8th, 1938.

† Obtainable from the Shaw Supply Company, Portland, Oregon.

about one-twentieth of an inch in diameter, giving a volume of 29.7 cubic millimeters for one inch on the $B'$ micrometer. The size of the plunger can be chosen for any ratio or volume desired, using the formula for volume of a cylinder to determine the proper radius. The small hole, $F$, is drilled to permit egress and ingress of air, and two holes for the set screws, $G$ and $H$, which hold the micrometers rigidly in place, are drilled and threaded. The set screws should be long enough to project above the steel block and permit rigid wiring of the micrometer to the ring stand.

The plunger of $B$ and the small steel rod, $C$, are greased. The chamber in the steel block is filled with clean mercury, the block is wired to the ring stand over a piece of flat rubber, and the stand with the attached block is jarred in various positions to force out any air bubbles.

To calibrate the apparatus, adjust the micrometer, $B$, to the one inch mark and attach (in the position shown in figure 2 for bulb, $I$) a mercury filled, right

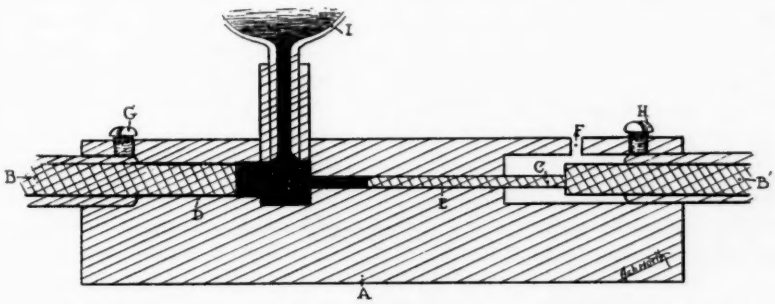


FIG. 1. DETAIL OF THE STEEL BLOCK WITH ATTACHED PARTS
The lettering is explained in the text

angled piece of Pyrex tubing with the end drawn out to a fine tip and turned down. The right angle should be close to the steel block. Manipulate $B$ until the tubing is free from air bubbles. The knurled heads of the micrometers are wound with electricians' tape to minimize conduction of heat from the fingers. Allow the apparatus to come to temperature equilibrium by standing 12 hours or longer in a room of relatively constant temperature. Calibrate the full inch of $B$ by several weighings on fine balances of the amount of mercury delivered into, or aspirated from a tared weighing bottle containing a small excess of mercury which is touched to the fine glass tip. During aspiration or ejection of the mercury, record the temperature of the room in the vicinity of the apparatus or, better, of the mercury itself with a thermometer accurate to 0.1°. Calibrate the volume equal to one inch on the coarse micrometer, $B$, from tables of the density and volume of mercury such as are given in any Handbook of Chemistry and Physics. For the micrometers we have used, this volume has proved to be 803 cubic millimeters. The fine microm-

eter, $B'$, is calibrated against the coarse micrometer, $B$, after the apparatus has been assembled.

A bulb, $I$, is blown from Pyrex glass tubing and fused to a Pyrex three way stop-cock as shown in figure 2, and the Pyrex tubing leading from this stop-cock is cut off and bent as shown in figure 2. The apparatus is then assembled as shown in figure 2. The bulb with the stop-cock is attached, making certain that the end of the glass does not extend below the end of the pressure tubing or

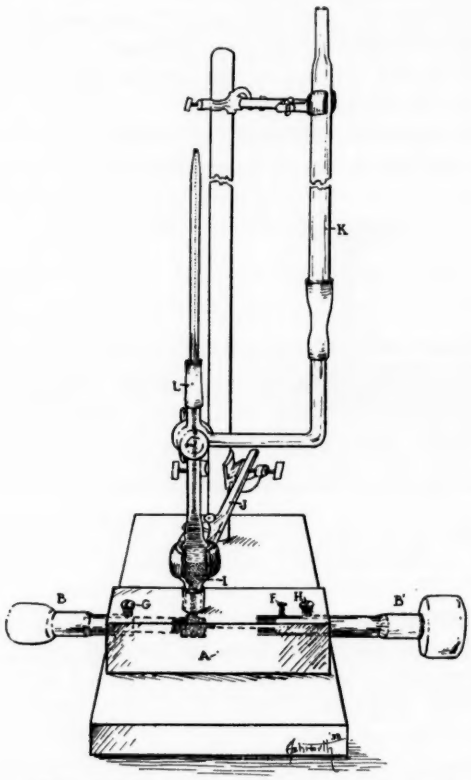


FIG. 2. ASSEMBLY OF THE APPARATUS

the tubing below the edge of the steel, so that no bubbles of air can be trapped. The bulb is clamped rigidly by clamp, $J$, and mercury is introduced by alternately turning in and out micrometer, $B$, until the bulb is filled with mercury to its mid-portion. The mercury is again jarred to be sure it is free of bubbles. The reservoir (a 10 cc. Mohr pipette is convenient), filled with water containing a dye, such as fuchsin, is attached as shown in figure 2, and the remainder of the bulb is filled with this dye solution. The reservoir may be covered by a test

tube to keep out dust. Turn the stop-cock so that it connects $K$ and $I$ and aspirate fluid into the bulb by turning micrometer $B$, then turn the stop-cock to the position shown in the illustration and force out the air through $I$. Repeat this process until the fluid fills all channels and there are no air bubbles. Heavy pressure tubing or a rubber stopper drilled to fit is attached and the pipettes to be calibrated are inserted up-side down. The end of the pipettes should almost touch $L$.

To calibrate the fine micrometer, $B'$, select a pipette of such size that two widely spaced marks on it will correspond to slightly less than 1.0 inch on $B'$. With the stop-cock in the position shown in figure 2, bring the meniscus of the fluid to the lower of these marks with $B$, while $B'$ is at the one inch mark. All readings of menisci should be made with a 5 to 10 $\times$ hand lens. Read $B$ to four decimal places and bring the fluid to the upper mark by use of $B$, and record the reading. Repeat this process several times, cleaning and drying the pipette between each calibration. Determine the average of the differences between these readings. Repeat the measurement, using the $B'$ micrometer exclusively. Determine the volume of the pipette between these marks by multiplying the average difference in readings recorded for $B$ by the capacity for one inch as previously determined. Divide this volume by the difference in readings on $B'$ to get the volume in cubic millimeters equal to one inch on $B'$. Record these figures in a convenient position on the apparatus. If it is desired to test the uniformity of the plungers select a pipette or capillary tube with two marks placed at such a distance apart that they represent 0.1 to 0.2 inches on the plunger to be tested, and take readings of this volume over the entire range of the micrometer.

In calibrating pipettes to deliver between two marks, fill the pipette first by turning the stop-cock so that it communicates with the reservoir, $K$. Then adjust to the desired mark and draw the fluid back with the micrometer. In calibrating to contain, the pipette should be clean and dry. Force the fluid from the lower mark to the upper mark with a micrometer. In calibrating hemocytometer pipettes, use the large plunger for the bulb and the small plunger for the measurement from the 0.5 mark to the tip. It has been found that while many Bureau of Standard hemocytometer pipettes are accurate at the 1.0 mark, they may have appreciable errors at the 0.5 mark which is the one almost always used in actual counting. To calibrate pipettes of a capacity larger than one inch on the micrometer, run the micrometer through its full range and then turn the stop-cock so it connects $K$ and $I$ and aspirate a fresh supply of fluid, repeating as often as necessary.

Precautions necessary for accuracy are that there be no trapped air bubbles, that the apparatus be in temperature equilibrium, and that there be no leaks. Air bubbles large enough to cause errors are readily visible. If there is a leak or the temperature is changing too rapidly, the fluid level will alter from the mark on a Sahli pipette within a minute or two. To secure the maximum accuracy of which the instrument is capable on long pipettes of small capacity, such as a

Sahli pipette, a correction factor for the elastic expansion of the steel and glass chamber per one inch column of water should be determined. To determine it, connect the top of a Sahli pipette in position in the instrument by rubber tubing to a water filled manometer. Adjust the fluid level in the pipette to the mark. Read the $B'$ micrometer. With a blood pressure bulb, raise the water level 10 inches in the manometer and readjust the meniscus to the mark with micrometer $B'$, and record the reading. The difference between the two readings, multiplied by the volume factor for $B'$, and divided by 10 is the desired factor. It is of the order of 0.15 c.mm. per one inch of water pressure. To use it, measure the distance between the points calibrated on any pipette, multiply the factor by this distance, and subtract the result from the volume determined from the micrometer readings.

The method has several advantages over any previous method of pipette calibration. It requires only a few seconds to calibrate one pipette. It is possible to adjust the menisci with great accuracy under adequate magnification. The presence of the bulb assures an almost constant pressure within the apparatus since the mercury level scarcely changes at all. The three way stop-cock permits rapid filling of the pipette from the reservoir and calibration of pipettes of greater capacity than the capacity of the large plunger. Slow temperature variations have very little effect because the only significant error introduced by temperature is when the temperature varies sufficiently to cause significant change in volume in the time between reading the upper and lower mark on the same pipette. The period of time necessary for these two readings is so short and the heat capacity of the apparatus is so great that if reasonable care is taken to protect the instrument from drafts, such errors are negligible. It may be used for checking calibration on pipettes or for placing calibrations on new pipettes. In the latter instance, it is convenient to cut rings of rubber tubing and slide them to the meniscus after rotating the micrometer to the calculated position. The instrument may be used as a microaspirator by substituting for the pipette a piece of right-angled glass tubing connected by rubber tubing to a very fine capillary tube handled by a micromanipulator. With this, it is easily possible to aspirate one leukocyte or other small particle under microscopic control. An instrument of this principle may be readily designed for any desired capacity by changing the size of the plunger.

## CONCLUSION

A simple accurate pipette calibrator and microaspirator has been described and illustrated.

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# A HOMEMADE ELECTRIC BONE SAW*

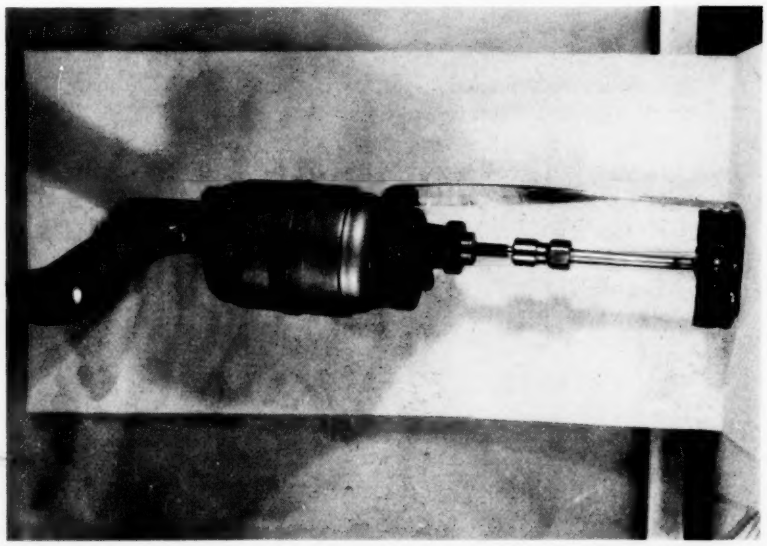
I. MILTON WISE

In 1931 I made the first model of an electric saw to facilitate the removal of the calvarium at autopsy. The first model used a high speed electric motor of $\frac{1}{8}$ H.P. with an extension fixed to the armature holding a small circular saw blade. This saw though efficient had several features that were objectionable; the speed was too great and the power of the motor too little so that it would frequently jam and stop.

The saw illustrated is the second model that has proved successful. It can be made for about $12.00 or $15.00. The power is derived from a K & M electric drill that retails for $10.00. The chuck is removed, and the threaded piece of the drill to hold the chuck is turned down to $\frac{1}{4}$ inch and a flat side filed on it. In the end of a tapered rod a $\frac{1}{4}$ inch hole is drilled and it is tapped for two set screws to fasten it to the drill end. The rod should be at least four inches long and the small end threaded to hold the Albee nuts so that a no. 012 Simmons Saw can be firmly

* Received for publication October 17th, 1938.

affixed. The guard is then soldered to a rod that fastens under one of the nuts on the housing of the motor. This can be easily removed when it becomes necessary to change the saw blade. The fastening nuts used in the Albee Bone outfit are made use of in securing the saw blade to the shaft, a guard is made of tin



and fastened to the housing of the motor which catches the bone fragments as well as serving as a protection for the pathologist. Because of the construction of the drill in the pistol grip type and the switch control on the handle the whole saw becomes very easy to handle and convenient to use.

## A PERMANENT METHOD OF LABELLING MICROSCOPE SLIDES*

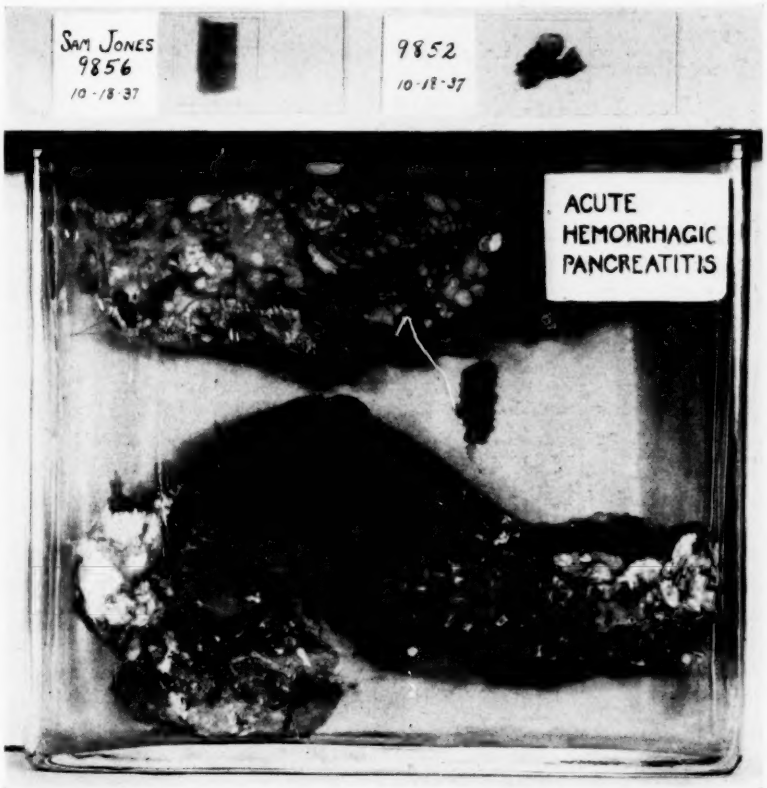
HAMILTON R. FISHBACK AND AVIS GREGERSEN

*From the Department of Pathology, Northwestern University Medical School*

The following method of labelling microscopic slides has been gradually evolved and is now in use.

* Received for publication October 8, 1938.

After mounting tissues or other material the blank end of the slide is wiped off with ether. Data can then be written on the glass with India ink, or stamped on it. After the ink dries the data space is brushed over quickly and lightly with clear lacquer* applied with a camel's hair brush. For a contrast background the reverse of the above space is covered with white lacquer.




It dries in a few minutes. A special lacquer thinner is used to keep the lacquer at proper consistency, and to wipe off any excess.

Lantern slides may be labelled similarly, or arrows inserted for regional emphasis, with India ink covered by the clear lacquer.

* The lacquer used at present is that of Rogers Co.

Reagent bottles, or other glass vessels, require a primary background coat of white lacquer put on the label area, and upon this the legend is stamped, then covered by clear lacquer. India ink written upon the white lacquer tends to crack as it dries.

## PUBLISHED PROCEDURES RECOMMENDED FOR TRIAL

### DETERMINATION OF HIPPURIC ACID IN URINE

T. E. Weichselbaum and J. G. Probstein, J. Lab. & Clin. Med., **24:** 636. 1939

The authors found that Quick's method for the estimation of hippuric acid in the urine as a liver function test has two merits, it is simple and it gauges the ability of the liver to conjugate benzoic acid and glycerine but it gave inaccurate and erratic results.

They claim for their modification of the test that it is more accurate, speedier, and allows the analysis of 150 cc. of urine without preliminary concentration.

*Method*

1. Measure the volume of the urine specimen and if over 150 cc. add a few drops of glacial acetic acid and evaporate to a volume not in excess of 150 cc.
2. Add 30 grams of sodium chloride per 100 cc. of urine, heat with shaking until the salt is dissolved.
3. Cool to about 15°–20°C. by immersing the flask in ice-cold water.
4. Add 1.2 cc. of approximately 10 N sulfuric acid and scratch the sides of the flask with a glass rod to enhance the crystallization of the hippuric acid. (This is important.)
5. Allow to stand fifteen minutes in the cold water, then filter through a Hirsch funnel (diameter of perforated plate 47 mm.), using moderate suction.
6. Wash the precipitate with chilled 30 per cent sodium chloride from a wash bottle, using the washing fluid first to rinse the flask. The precipitate is adequately washed when the washing fluid is free of sulfuric acid.
7. Transfer the funnel with its contents onto the flask which still contains some of the crystals and rinse the hippuric acid crystals into it by dissolving in hot water from a fine tipped wash bottle.
8. Heat until all crystals are dissolved and titrate with 0.5 N sodium hydroxide with phenolphthalein as an indicator.

*Calculation*

Multiplying the number of cubic centimeters of the 0.5 N sodium hydroxide by 0.072 gives the amount of sodium benzoate in grams from which the hip-

puric acid was derived. If to this one adds the correction for the solubility of hippuric acid (0.123 grams per 100 cc. of urine) in terms of sodium benzoate, i.e., 0.123 by 0.804 = 0.10 gram sodium benzoate one obtains the sodium benzoate value with a maximum error of ±10.5 mgm. per 100 cc.

If the urine specimens are dark colored or contain much bile the analysis is difficult but this difficulty can be overcome by adding 0.3 gram of acid washed norit per 100 cc. of urine, boil for one minute, cool, filter by suction on a Hirsch funnel and wash the residue with a small amount of hot water; measure the volume and proceed as above.

If the urine contains protein, it must be completely removed. The authors recommend the following method for this purpose. Measure the sample, add 5 cc. of 20 per cent copper sulfate solution, mix, then add with shaking 5 cc. of N/1 sodium hydroxide per 100 cc. of urine. Shake and heat to near boiling temperature, cool, filter through a good grade of filter paper into a graduated cylinder and record the volume. Proceed as before but take into account the dilution with the protein precipitants.

## MAKING OF GOLD SOLUTION FOR CEREBROSPINAL FLUID TESTS

B. S. Levine, Ven. Dis. Inf. **20:** 41. 1939

*Reagents:*

| | |
|---|---|
| Distilled water | |
| Gold chloride | 1 per cent |
| Sodium citrate | 1 per cent |
| Hydrogen peroxide (fresh) | 3 per cent |

*Method:* Heat 500 cc. of the water to brisk boiling, add 5 cc. of the gold chloride solution and shake for a few seconds. Add 25 cc. of the sodium citrate solution and shake for 10 or 15 seconds. While shaking add 5 minims of the hydrogen peroxide diluted with 10 cc. of distilled water. Continue shaking for a few seconds, allow the flask to rest on the table until the reaction is complete, this usually takes place in ½ to 1 minute.

Multiple or proportional fractions of the above volumes may be used.

The author claims the following advantages for his method: simplicity, reliability, and speed of preparation.

## A NEW SPINAL FLUID REACTION FOR THE DIAGNOSIS OF SYPHILIS

K. Mezey, Wien. Klin. Woch., **51:** 1058. 1938

(Abstract in Ven. Dis. Inf., **20:** 53. 1939)

The test is based on the detection of tryptophan since this substance has been found to be increased when there is destruction of the parenchyma of the central nervous system.

The method consists of adding a few drops of Ehrlich's reagent (p-dimethyl-

amino-benzaldehyde) to cerebrospinal fluid acidified with concentrated hydrochloric acid. Depending on the concentration of tryptophan a blue color is developed in 12 to 24 hours.

For a standard solution a 2 mg. per cent solution of tryptophan (acidified with HCl) was found suitable. For quantitative determination 1 cc. of the cerebrospinal fluid is placed in a small beaker and 3 cc. of concentrated HCl and 3 drops of Ehrlich's Reagent are added. For the control 1 drop of the reagent is added to 1 cc. of the standard tryptophan solution. The result is read colorimetrically after 24 hours.

The tryptophan values in cases of neurosyphilis vary between 0.1 and 1.5 mgm. per cent.

### SULFOCYANATE AS A TREATMENT FOR MANGE ON DOGS AND OTHER LABORATORY ANIMALS

E. B. Carmichael, J. Lab. & Clin. Med., **24:** 656. 1939

The author has been using, with success, a preparation of lauryl sulfocyanate called Loro and put out by E. I. du Pont de Nemours & Co. The preparation contains about 50 per cent of active ingredients and an emulsifying and wetting agent which allows one to use it in an aqueous solution. A 5 per cent aqueous solution is applied by means of a sponge. Washing dogs previous to the treatment is better but not absolutely necessary. It is not essential to treat the whole body if there are only a few small areas affected. The animal should be prevented from licking the treated part by means of a muzzle or holding for 30 minutes.

If the disease is of long standing and there are open sores or ulcers, more than one treatment may be necessary. If two or more treatments are used they should be given a week to ten days apart.

The treatment has been effective in guinea pigs, cats, rats, and dogs.

## ANNOTATIONS, MINOR CONTRIBUTIONS, QUERIES

### A PATHOGENIC AGENT IN ACUTE RHEUMATIC FEVER

In Science, **89:** 271, March 24, 1939, Homer F. Swift and Thomas McPherson Brown report some interesting experiments in which they were able to produce characteristic lesions by inoculating the chorioallantoic membranes of chicken eggs with exudates obtained from patients with acute rheumatic fever, which did not appear when similar membranes were inoculated with non-rheumatic exudates.

Mice inoculated intranasally with rheumatic exudates and with suspensions of chorioallantoic membranes showing the lesions described above develop pneumonia. That suspensions of these lungs contain the inciting agent (which incidentally passes through Berkefeld V candles) is shown by the fact that it is

transmissible from series to series by using as inocula ground pneumonic lungs suspended in broth, and ordinary bacteria are absent both from films and cultures.

Using the methods of Sabin they were able to grow pleuro-pneumonia-like organisms from the pneumonic mouse lungs and from the chorioallantoic membranes, furthermore by using the same methods similar organisms were grown from arthritic exudates.

Intranasal inoculation of mice with subcultures of the organism has produced a pneumonia similar to that produced by inoculation with rheumatic exudates and with suspensions of chorioallantoic membranes infected with rheumatic exudates.

## CANCER TESTS

For years investigators have sought a laboratory method whereby cancer in the human body may be detected without resorting to operation procedures, indeed many tests have been evolved and advocated but up to the present time none has met with general acceptance. Pathologists are properly skeptical of all such tests and some have, on philosophical grounds, doubted the possibility of such a test. For the more hopeful it is recommended that they read the short article entitled A Selective Action of Urine and Serum from Patients with Malignant Tumors on Embryonal and Newly Growing Tissues, by THEODORE H. ELSASSER AND GEORGE B. WALLACE, in Science, **89**: 250, March 17, 1939.

These observers have found that pregnant rabbits injected with urine or blood serum from patients having malignant tumors abort in about five days while similar animals do not abort when the urine or serum of normal individuals or pregnant women is injected.

They find interesting changes in the uterus of the pregnant animals, also in the ovaries of non-pregnant females and the testicles of males.

The authors do not suggest the obvious possibility of using the procedure as a clinical test for malignancy but merely state that work is being continued on the many interesting problems which arise in connection with it.

## PHOTOGRAPHIC TECHNIC

*To the Editor:*—I noticed on the inside cover of the TECHNICAL SUPPLEMENT under Annotations, Minor Contributions, Queries that it says "an attempt will be made to obtain answers from authoritative sources to queries submitted," and wondered if you could help us.

We are working on Equine Periodic Ophthalmia and on a horse disease commonly known as "Wobbles" which gives a clinical picture similar to Locomotor Ataxia in humans.

We are anxious to begin taking our own pictures and would like, if possible, to get a camera with which we could take the following types of pictures—

Close-ups of the eyes of live horses, close-ups of pathological specimens, eyes and bones, with an attachment to cut down the high-lights, full-figure pictures of "Wobblers," photomicrographs, close-ups taken through an ophthalmoscope, if possible.

We would also like to know the title of a Handbook of Photography for Amateurs.

F. P.

*Answer:*—The above query was submitted to Mr. A. Ter Louw of the Eastman Kodak Company who replied as follows: "The problem placed before you relative to photographing the various manifestations of equine periodic ophthalmia is a very interesting one, and from our experience we believe that a camera similar to the Kodak Recomar would be the most satisfactory for your work. This is one of the most versatile cameras of moderate price with which we are familiar, and we have used it successfully for general speed work such as would be involved in photographing the animal as a whole. We have used it with Pola-Screens for photographing pathological specimens, and we have used it in connection with the microscope for making photomicrographs. Its double bellows extension permits photographing at almost actual size so that excellent close-ups of the eye could be obtained.

Although slightly more expensive, the reflex feature of the Revolving Back Auto Graflex (either $3\frac{1}{4}''$ x $4\frac{1}{4}''$ or $4''$ x $5''$) presents definite advantages in this work. Where absolute immobilization is impossible, as in photographing the eye of the horse, it may be found that the added cost of this camera will be amply repaid by the reduction of the number of failures due to unsharp focus and movement of the subject.

Photography through the ophthalmoscope is a slightly different matter. To our knowledge, the only really successful method for photographing the background of the eye is by means of special apparatus designed primarily for this purpose. The one most commonly used in this country is the Nordensen Reflex-free Retinal Camera distributed by Carl Zeiss, Inc., 485 Fifth Avenue, New York City.

This camera was designed for use on humans, and its application to photography of the background of the eye of a horse may present difficulties on account of the size of the instrument. We are not familiar with anyone who has successfully photographed the background of the eye through an ordinary hand ophthalmoscope, such as is commonly used for inspection.

Incidentally, with regard to photographing pathological specimens through Pola-Screens we might point out that the complete elimination of specular highlights is not always desirable. Many times we have found that a photograph of a pathological specimen in which the highlights are kept at a minimum size by using small light sources at a distance of 5 to 6 feet and the lights are so placed that the highlights do not fall on the important areas is preferred to one in which Pola-Screens have been used. Nevertheless, we are enclosing a memorandum on the use of Pola-Screens in photographing pathological specimens in the event it is felt that this feature is desirable.

We are enclosing a short bibliography of books which might be used by the amateur in getting started in this work.

### Photography of Pathological Specimens with Pola-Screens

In many instances the small, glaring highlights characteristic of wet surfaces do not interfere with the usefulness of a photograph of a pathological specimen, especially if they are kept small in area and the lights are so arranged that they fall in a position where they do not hide important detail. Sometimes, however, it is desirable to eliminate these highlights completely or at least reduce their intensity. The most efficient way of doing this is through the use of polarized light. The equipment necessary is as follows:

One Type 1A Pola-Screen to fit over the camera lens.

Two Eastman Pola-Screens IIB with stands to polarize the light falling upon the specimen.

In use, the lights are placed in approximately the same position as in ordinary photography of subjects without wet, shiny surfaces. A Type IIB Pola-Screen is placed before each lamp in such a manner that all the light falling upon the specimen passes through the screens. The Type 1A Pola-Screen is placed over the camera lens. The two Type IIB Pola-Screens should be aligned so that their polarizing axes are parallel. This is done in the following manner:

The right-hand light is turned on and the Type 1A Pola-Screen on the lens is rotated until the specular highlights are eliminated from the image on the ground-glass. This light is then turned off and the left-hand light is turned on. By means of the ball joint on the stand, the Type IIB Pola-Screen before this lamp is rotated on an axis formed by a line between the light source and the specimen until a position is found in which the specular highlights produced by this light source are eliminated from the image on the ground-glass. Now both lights can be turned on and the picture will be free from specular highlights.

With most subjects, an exposure of 16 to 20 times that given without the use of Pola-Screens is required. Many times it will be found that a photograph made with the screens in this position will appear dead and lifeless. This can be eliminated to a certain extent by rotating the Type 1A Pola-Screen before the lens until specular highlights are just visible in the ground-glass image. Such a picture will give the impression of a wet, shiny surface, but the highlights will not be strong enough to hide important detail. When the orientation of the screens deviates from the "crossed" position in this manner the exposure increase is not as great as mentioned above. In any event, the exposure factor cannot be reduced below 4 times.

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